Renal Dendritic Cells Ameliorate Nephrotoxic Acute Kidney Injury

Raghu K. Tadagavadi* and W. Brian Reeves[†‡]

*Department of Biochemistry and Molecular Biology and [†]Division of Nephrology, Penn State University College of Medicine, Hershey, Pennsylvania; and [‡]Lebanon VA Medical Center, Lebanon, Pennsylvania

ABSTRACT

Inflammation contributes to the pathogenesis of acute kidney injury. Dendritic cells (DCs) are immune sentinels with the ability to induce immunity or tolerance, but whether they mediate acute kidney injury is unknown. Here, we studied the distribution of DCs within the kidney and the role of DCs in cisplatin-induced acute kidney injury using a mouse model in which DCs express both green fluorescence protein and the diphtheria toxin receptor. DCs were present throughout the tubulointerstitium but not in glomeruli. We used diphtheria toxin to deplete DCs to study their functional significance in cisplatin nephrotoxicity. Mice depleted of DCs before or coincident with cisplatin treatment but not at later stages experienced more severe renal dysfunction, tubular injury, neutrophil infiltration and greater mortality than nondepleted mice. We used bone marrow chimeric mice to confirm that the depletion of CD11c-expressing hematopoietic cells was responsible for the enhanced renal injury. Finally, mixed bone marrow chimeras demonstrated that the worsening of cisplatin nephrotoxicity in DC-depleted mice was not a result of the dying or dead DCs themselves. After cisplatin treatment, expression of MHC class II decreased and expression of inducible co-stimulator ligand increased on renal DCs. These data demonstrate that resident DCs reduce cisplatin nephrotoxicity and its associated inflammation.

Innate immune responses are pathogenic in both ischemic and toxic acute renal failure. In response to renal injury, inflammatory chemokines and cytokines are produced both by renal parenchymal cells, such as proximal tubule epithelial cells, and resident or infiltrating leukocytes.[1–4] The elaborated chemokines and cytokines, including TNF-α, IL-18, keratinocyte-derived chemokine, and monocyte chemoattractant protein 1, subsequently recruit additional immune cells to the kidney, such as neutrophils, T cells, monocytes, and inflammatory dendritic cells (DCs), which may cause further injury through pathways that are not fully defined.[2,5–12] DCs are sentinels of the immune system and under steady-state conditions induce tolerance by various mechanisms, including production of TGF-β, IL-10, or indoleamine 2,3-dioxygenase[13–16]; expression of PDL-1, PDL-2, or FcγR2B[17,18]; clonal deletion of autoreactive T cells[19]; and induction of T regulatory cells *via* the inducible co-stimulator (ICOS) pathway.[20–23] In response to pathogens or products of tissue injury, DCs mature and initiate immunity or inflammatory diseases.[24,25] Monocytes recruited to inflamed tissue can also differentiate into inflammatory DCs and mediate defense against pathogens or contribute to inflammatory tissue responses.[12,26–28]

Although DCs represent a major population of immune cells in the kidney,[29] their role in renal disease is poorly defined. Liposomal clodronate has been used to study the pathophysiologic role of phagocytic cells,

which include DCs and macrophages.[3,30–32] An alternative DC-specific approach uses expression of the simian diphtheria toxin receptor (DTR) driven by the CD11c promoter to target DCs for DT-mediated cell death.[24] This model has been used extensively to study the role of DCs in various physiologic and pathophysiologic contexts[32,33]; however, its application in kidney disease has been limited to recent studies of immune complex–mediated glomerulonephritis.[12,23]

We have reported that inflammation plays an important role in the pathogenesis of cisplatin-induced acute kidney injury (AKI).[1,4,5,34] Given the dearth of information regarding the role of renal DCs in AKI, this study examined the renal DC population and the impact of its depletion on cisplatin nephrotoxicity. We show that DCs are the most abundant population of renal resident leukocytes and form a dense network throughout the tubulointerstitium. Renal DCs displayed surface markers that distinguished them from splenic DCs. Using a conditional DC depletion model, we determined that DC ablation markedly exacerbates cisplatin-induced renal dysfunction, structural injury, and infiltration of neutrophils.

RESULTS

Properties of Renal DCs

Flow cytometric analysis of tissue digests revealed a distinct population of cells expressing high levels of CD11c and MHC class II (MHCII) in both kidney and spleen (Supplemental Figure 1A). On the basis of this definition, DCs were the most abundant population of leukocytes in the kidney ($46.15 \pm 2.5\%$ of all $CD45^+$ cells; $n = 4$). A total of $9.4 \pm 0.6 \times 10^4$ $CD11c^+$ $MHCII^+$ DCs were obtained from each kidney compared with $6.8 \pm 0.3 \times 10^5$ from the spleen of normal C57BL/6 mice ($n = 12$). Comparative analysis of cell surface markers of renal and splenic DCs (Supplemental Figure 1B) showed similar expression of CD11b, MHCI, MHCII, CD40, CD80, CD86, and Gr-1. In contrast to splenic DCs, renal DCs were negative for CD4 and CD8. Renal DCs also differed from the bulk of splenic conventional DCs by their prominent expression of F4/80, a surface marker of monocytes and macrophages (Supplemental Figure 1, B and C).

We used transgenic mice (CD11c-DTRtg) that express green fluorescence protein (GFP) and the simian DTR driven by the CD11c promoter to examine renal DC distribution (Figure 1, A and C through F). Confocal imaging revealed GFP-positive cells throughout the tubulointerstitium but not in glomeruli (Figure 1, E and F). GFP-positive cells had a stellate shape typical of DCs. As expected, wild-type (WT) mice showed no GFP positive cells (Figure 1B).

Inducible Ablation of Renal DCs In Vivo

To deplete selectively DCs *in vivo*, we used CD11c-DTRtg mice in which expression of the DTR/GFP fusion protein in DCs renders the normally resistant murine cells sensitive to DT-
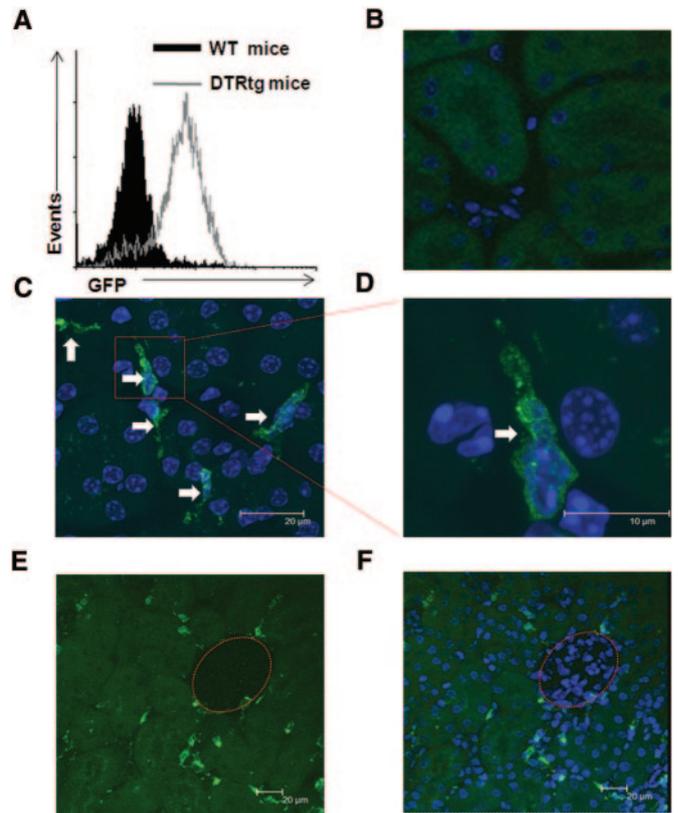

Figure 1. DCs in the kidney are shown. (A) Flow cytometry of renal $CD11c^+$ $MHCII^+$ DCs from WT and CD11c-DTRtg mice for GFP expression. (B through F) Confocal microscopy of kidney sections of WT (B) and CD11c-DTRtg mice (C through F) for GFP-positive DC localization. (D) Magnification of boxed area in C showing GFP-positive cells between renal tubules. (E and F) Absence of GFP-positive cells (DCs) in glomerulus. Arrows point to GFP-positive DCs. Nuclei are stained with DAPI.

induced cell death.[23–25] We first examined the efficiency of depletion of renal and splenic DCs in response to DT treatment. Similar to the recent study by Scholz *et al.*,[23] DT administration to CD11c-DTRtg mice caused a marked transient depletion of $CD11c^+$ $MHCII^+$ cells in both kidney and spleen (Figure 2). Ablation was maximal at 24 h in spleen and at 48 h in kidney (Figure 2, C and D). DC depletion in the kidney was more prolonged than in spleen. DT did not deplete $CD11c^-$ $F4/80^+$ macrophages in the kidney (Supplemental Figure 2).

DC Depletion Exacerbates Cisplatin Nephrotoxicity

DT was injected 24 h before cisplatin injection to ablate DCs. Treatment of either CD11c-DTRtg mice or their WT littermates with DT alone did not result in renal dysfunction as assessed by levels of blood urea nitrogen (BUN) and serum creatinine (Figure 3). In comparison with WT mice, CD11c-DTRtg mice that were administered an injection of DT followed by cisplatin showed both earlier and more dramatic increases in serum creatinine and BUN. The survival at 72 h was only 20% in the CD11c-DTRtg mice and 100% in WT mice treated with DT and cisplatin ($n = 5$).
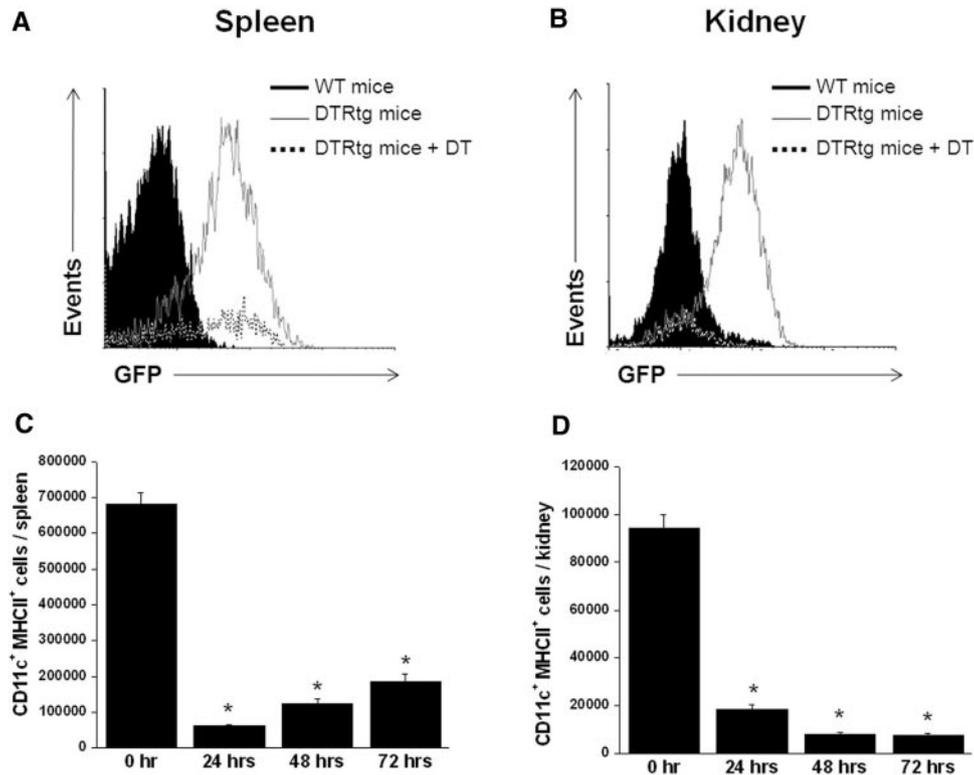
Figure 2. DC depletion in CD11c-DTRtg mice after DT treatment is shown. (A and B) Histogram of GFP expression by splenic (A) and renal (B) CD11c$^+$ MHCII$^+$ DCs in WT and CD11c-DTRtg mice and their depletion at 24 h after DT treatment. (C and D) Flow cytometric quantification of spleen (C) and kidney (D) CD11c$^+$ MHCII$^+$ DCs obtained from CD11c-DTRtg mice at various time points after DT injection. *$P < 0.05$ versus 0 h group ($n = 4$ to 12).
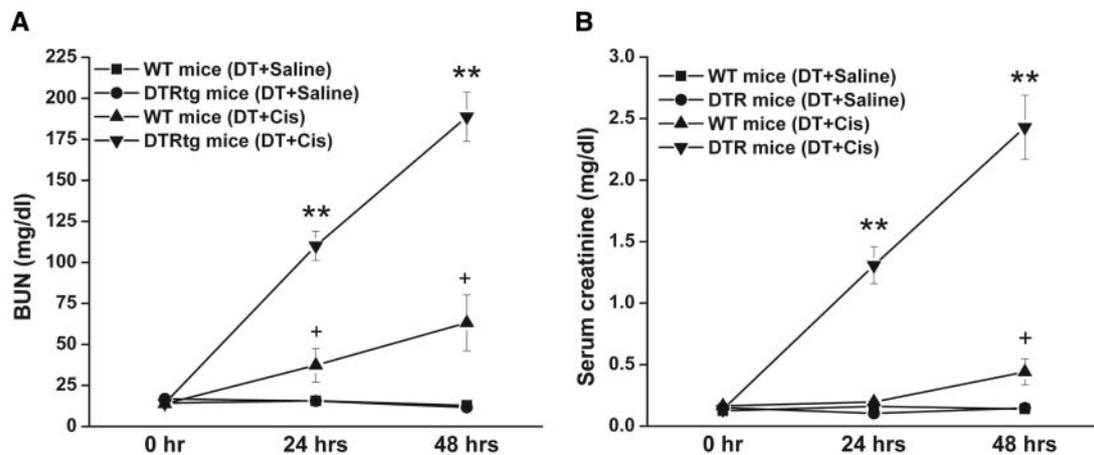

Figure 3. DC depletion increases susceptibility to cisplatin nephrotoxicity. WT and CD11c-DTRtg mice were treated with either DT or DT and cisplatin. (A and B) Blood collected at various time points with respect to cisplatin injection was analyzed for BUN (A) and serum creatinine (B) as a measure of renal function. **$P < 0.01$ versus all other groups; $^+P < 0.05$ versus all other groups ($n = 9$ to 17).

The worsening renal dysfunction in response to cisplatin treatment in DC-depleted mice was associated with an increase in tubular necrosis (Figure 4). Kidneys from DT-treated WT mice and CD11c-DTRtg mice did not exhibit any visible tubular damage (Figure 4, A, B, and E). WT mice treated with DT followed by cisplatin displayed moderate tubular injury characterized by dilation of tubules, loss of brush border, and sloughing of epithelial cells (Figure 4, C and E). CD11c-DTRtg mice treated with DT followed by cisplatin showed more extensive renal tubular damage compared with mice not depleted of DCs (Figure 4, D and E).

Tissue injury is associated with inflammation and infiltration of leukocytes.[5] Because DC ablation exacerbated renal dysfunction as early as 24 h after cisplatin injection, we inves-
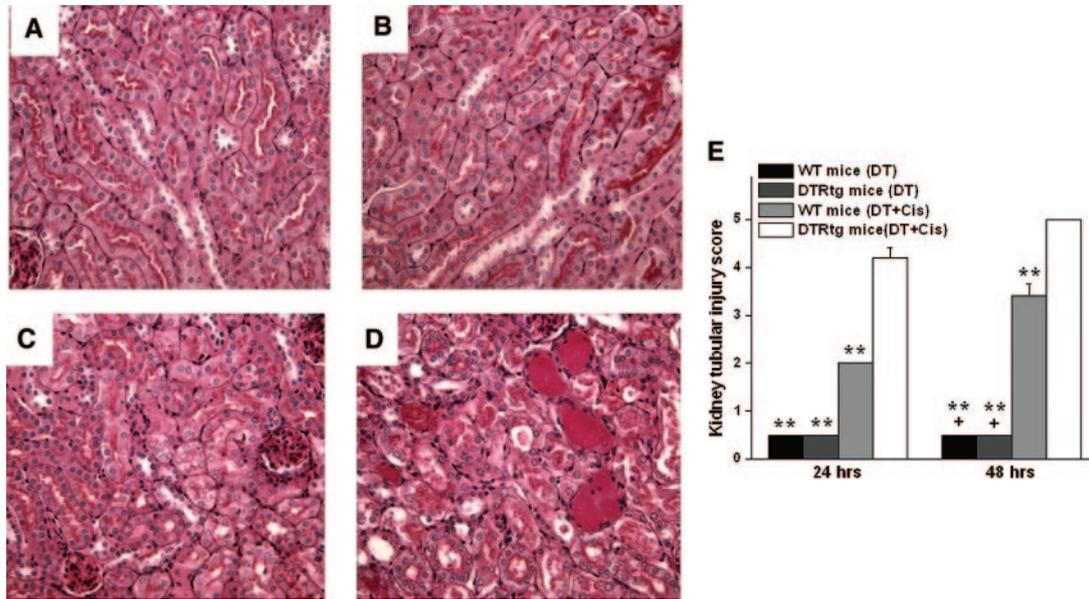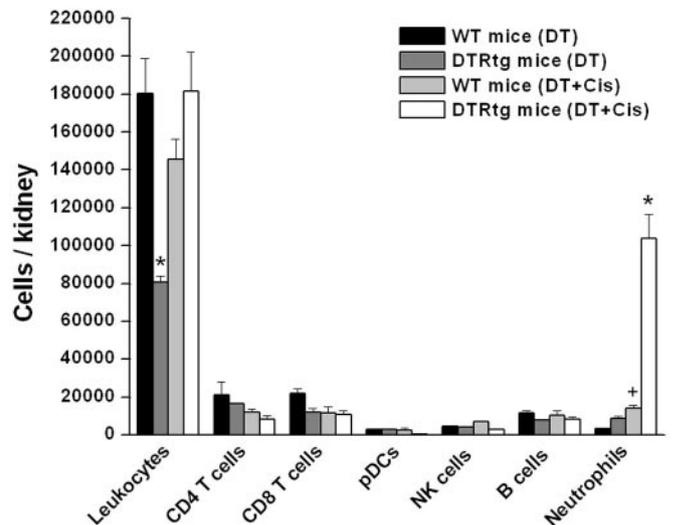
Figure 4. Effect of DC depletion on renal morphology in cisplatin nephrotoxicity is shown. (A through D) WT mice (A and C) and CD11c-DTRtg mice (B and D) were treated with DT (A and B) or DT and cisplatin (C and D). Cisplatin was injected 24 h after DT injection (C and D). Kidneys were harvested at 48 h after cisplatin injection and stained with periodic acid-Schiff. (E) Tubular injury scoring in the renal cortex of periodic acid-Schiff–stained kidney sections harvested at 24 and 48 h after cisplatin injection. $**P < 0.01$ *versus* CD11c-DTRtg mice (DT + Cis); $^+P < 0.05$ *versus* WT mice (DT + Cis) ($n = 4$ to 5).

tigated the influx of leukocytes into the kidney in WT and DC-ablated mice to cisplatin nephrotoxicity. DT-treated CD11c-DTRtg mice showed a significant reduction in total kidney leukocytes compared with DT-treated WT mice (Figure 5), which reflected the depletion of DCs themselves, which constitute a major leukocyte population in the kidney. Cisplatin treatment resulted in a striking increase in leukocyte infiltration in DC-depleted CD11c-DTRtg mice, with neutrophils constituting the major infiltrating leukocyte population. WT mice treated with cisplatin showed only a moderate increase in neutrophils. The number of T cells, B cells, NK cells, and plasmacytoid DCs infiltrating the kidney were not dramatically altered by either DT or cisplatin in either WT or CD11c-DTRtg mice. Immunohistochemistry for neutrophils confirmed the results obtained by flow cytometry (Figure 6).

We also examined the impact of the timing of DC depletion on cisplatin injury. WT and DTRtg mice were administered an injection of DT either 24 or 1 h before or 24 h after cisplatin injection (Figure 7). Injection of CD11c-DTRtg mice with DT either 24 or 1 h before cisplatin injection exacerbated renal injury to a similar extent, whereas injection of CD11c-DTRtg mice with DT 24 h after cisplatin injection had no impact on the severity of renal dysfunction (Figure 7). These results suggest that DC depletion at late stages does not influence the ongoing kidney injury in cisplatin nephrotoxicity.
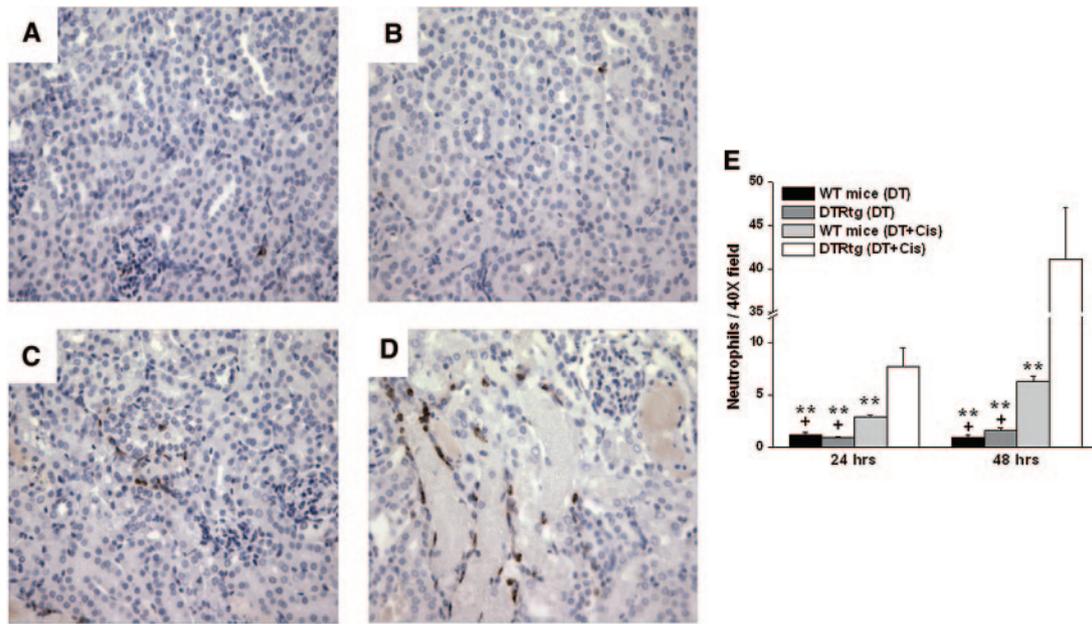
Effects of DT on Cisplatin Nephrotoxicity Are Mediated by Hematopoietic CD11c-Expressing Cells
To exclude the involvement of DTR expressed on nonhematopoietic cells in the previous results, we created chimeric mice in


Figure 5. Effect of DC depletion on renal leukocyte infiltration in cisplatin nephrotoxicity is shown. WT and CD11c-DTRtg mice treated with DT or DT and cisplatin were killed at 24 h, and single-cell kidney suspensions were analyzed by flow cytometry gating on CD45$^+$ leukocytes for total numbers of CD4$^+$ and CD8$^+$ T cells, CD11b$^-$PDCA-1$^+$ plasmacytoid DCs, CD3$^-$NK1.1$^+$ NK cells, CD11c$^-$B220$^+$ B cells, and F4/80$^-$Ly-6G$^+$ neutrophils per kidney. $*P < 0.05$ *versus* all other groups; $^+P < 0.05$ *versus* WT mice (DT) ($n = 4$ to 5).

which CD11c-DTR/GFP bone marrow was transplanted into irradiated WT recipients, thereby restricting DTR expression to the hematopoietic compartment (Supplemental Figures 3B and 9A). We also made WT to CD11c-DTRtg chimera to de-

Figure 6. Effect of DC depletion on renal neutrophil infiltration and localization in cisplatin nephrotoxicity is shown. (A through D) WT mice (A and C) and CD11c-DTRtg mice (B and D) were treated with DT (A and B) or DT and cisplatin (C and D). Cisplatin was injected 24 h after DT treatment (C and D). Kidneys harvested 48 h after cisplatin injection were stained for neutrophils. (E) Enumeration of neutrophils in renal cortex of kidney sections harvested at 24 and 48 h after cisplatin treatment. **$P < 0.01$ *versus* CD11c-DTRtg mice (DT + Cis); [+]$P < 0.05$ *versus* WT mice (DT + Cis) ($n = 4$ to 5).
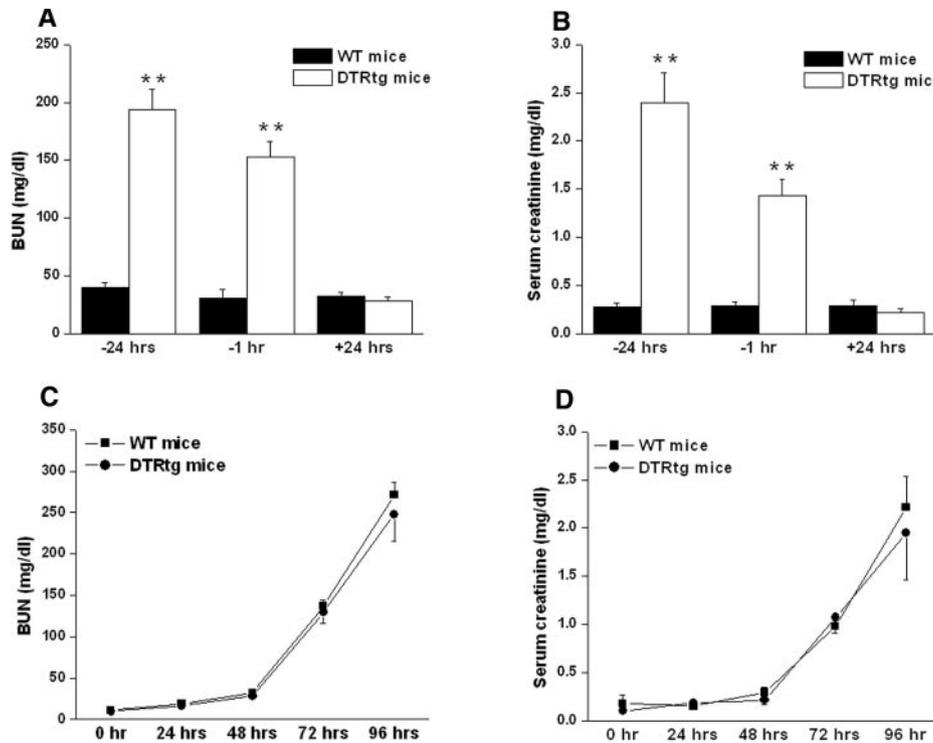
Figure 7. Kinetic analysis of renal DC role in cisplatin nephrotoxicity is shown. WT and CD11c-DTRtg mice were treated with DT at −24, −1, or 24 h with respect to cisplatin injection. (A and B) Blood collected at 48 h after cisplatin injection was analyzed for BUN (A) and serum creatinine (B). (C and D) WT and CD11c-DTRtg mice were administered an injection of cisplatin followed by DT 24 h later. Blood collected at various time points with respect to cisplatin injection was measured for BUN (C) and serum creatinine (D). **$P < 0.01$ *versus* WT mice ($n = 7$ to 10).

termine the efficiency of renal DC depletion in response to the irradiation protocol used to make the chimeric mice (Supplemental Figure 3A). The results indicate that the resident renal DCs in bone marrow chimeras are almost exclusively of donor origin.

Having established that the kidney DCs in CD11c-DTRtg to WT chimeric mice are of donor origin, CD11c-DTRtg to WT chimeric mice were administered an injection of DT and cisplatin. To ensure maximal depletion of DCs, the mice received DT 24 h before and 24 h after cisplatin injection.[32,33,35,36] Consistent with our previous observation in CD11c-DTRtg mice, the CD11c-DTRtg to WT chimeric mice depleted of DCs showed more severe renal dysfunction as measured by BUN (Figure 8A) and serum creatinine (Figure 8B) than nondepleted chimeric mice treated with cisplatin alone. Likewise, CD11c-DTRtg to WT chimeric mice treated with DT and cisplatin exhibited increased mortality compared with CD11c-DTRtg to WT chimeric mice that received cisplatin alone (Figure 8C). Treatment of CD11c-DTRtg to WT chimeras with DT alone did not produce any mortality.

Exacerbation of Injury by DT Is Independent of Dead DCs

Administration of DT to CD11c-DTRtg mice or CD11c-DTRtg to WT chimera results in massive death of DCs. The exacerbation of cisplatin nephrotoxicity by DT, then, could be the result of these dead DCs, perhaps serving as a danger signal to the immune system,[37] rather than the absence of DCs, *per se*. To address this possibility, we made mixed bone marrow chimeric mice in which 50% of the DCs were WT and 50% expressed DTR and were susceptible to DT-induced cell death (Figure 9A). Treatment of these mice with DT produced DC death but did not fully deplete DCs. DT was injected into these mixed chimeric mice 24 h before and 24 h after cisplatin treatment. DT treatment had no impact on the severity of cisplatin-induced renal dysfunction in the mixed chimeras as deter-

mined by levels of BUN (Figure 9B) and serum creatinine (Figure 9C). These results indicate that dead or dying DCs do not account for the worsening of cisplatin nephrotoxicity in DT-treated mice.

Lack of Inflammatory DCs in Cisplatin Nephrotoxicity

In nephrotoxic nephritis, CD11b^{hi}Gr-1^{hi}CD11c$^+$-expressing monocyte-derived proinflammatory DCs are recruited to the kidney.[12] We quantified CD11b^{hi}Gr-1^{hi}CD11c$^+$ DCs in the kidney after cisplatin treatment (Figure 10, A and C). WT mice that were administered an injection of cisplatin showed a very minimal infiltration of CD11b^{hi}Gr-1^{hi}CD11c$^+$ inflammatory DCs at 48 and 72 h compared with saline-treated mice; however, kidneys of cisplatin-treated mice showed a time-dependent increase in the number of CD11b^{hi}Gr-1^{hi}CD11c$^-$ cells. Gr-1 is a surface marker detected by mAb RB6-8C5 that binds to both Ly6C and Ly6G.[27,38] The 7/4 antibody detects a 40-kD antigen expressed on monocytes and neutrophils.[39] By morphology, neutrophils are Ly6G^{hi}7/4^{hi} and monocytes are Ly6G$^-$7/4^{hi}.[39] To characterize these infiltrating leukocytes further, we stained cells for Ly6G and 7/4 (Figure 10, B and D). We observed a time-dependent increase in cells positive for both Ly6G and 7/4, suggesting them to be neutrophils.[39] Likewise, the number of Ly6G$^-$7/4^{hi} monocytes[39] increased at 72 h in cisplatin-treated mice. Thus, neutrophils and monocytes, rather than inflammatory DCs, are increased in cisplatin nephrotoxicity.

Activation State of Renal DCs in Cisplatin Nephrotoxicity

Renal DCs from cisplatin-treated mice were examined for the changes in the expression of MHCI, MHCII, CD40, CD80, and CD86 (Figure 11A), the maturation markers that are required for the induction of inflammatory response, and ICOS-L (Figure 11, B and C), which can induce IL-10 production by T cells.[20–23] Kidney DCs from WT mice treated with saline or cisplatin showed
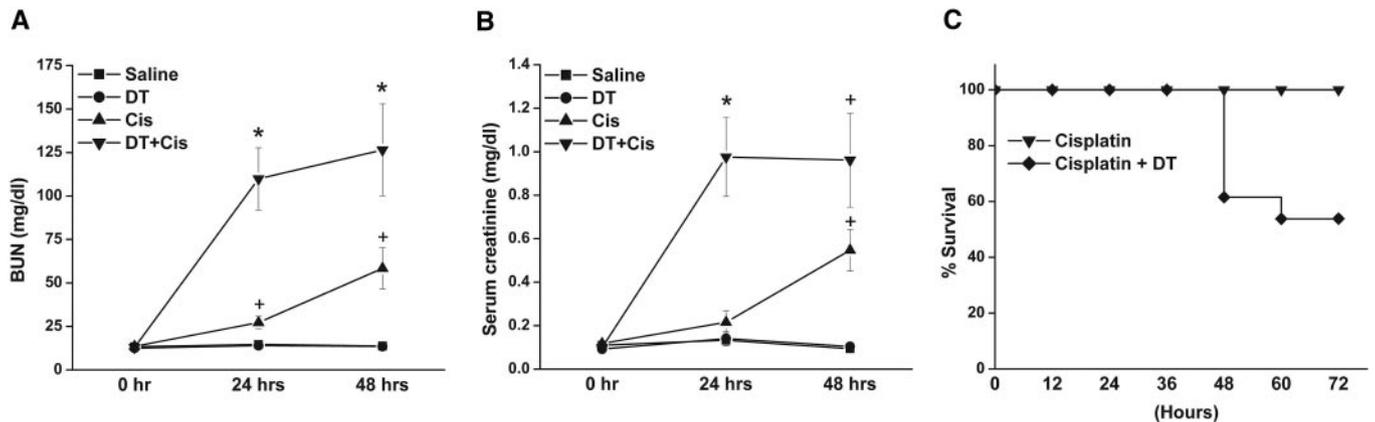
Figure 8. DC-mediated protection is independent of DT effect on nonhematopoietic cells. CD11c-DTRtg to WT chimera mice were administered an injection of saline, DT, cisplatin, or DT and cisplatin. (A and B) Blood collected at various time points with respect to cisplatin injection was analyzed for BUN (A) and serum creatinine (B). (C) Survival rate was determined in CD11c-DTRtg to WT chimera after the administration of cisplatin or DT and cisplatin (n = 8 to 13). $^+P < 0.05$ *versus* saline and DT; $^*P < 0.05$ *versus* all other groups.
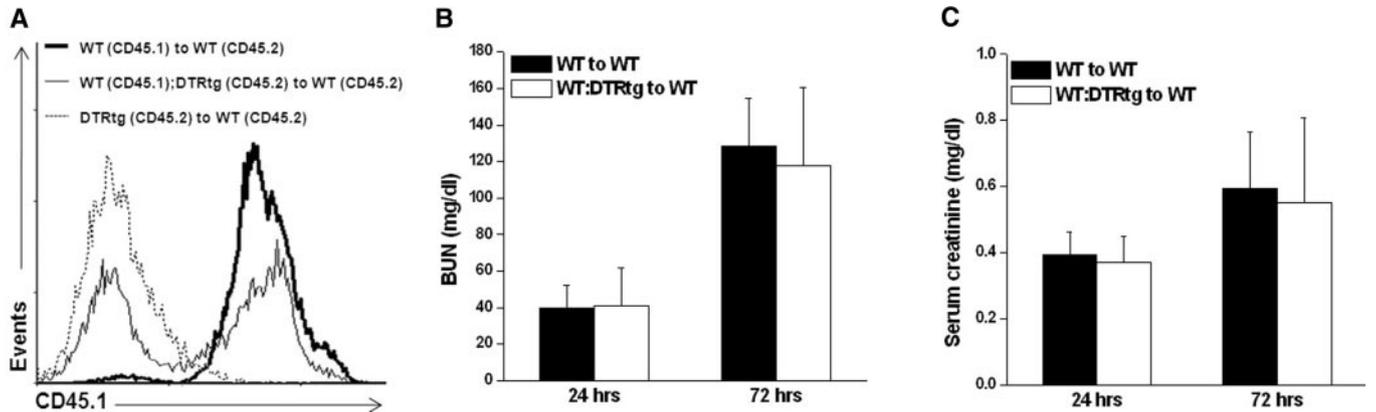
**Figure 9.** Effect of dead cells on DC-mediated protection in cisplatin nephrotoxicity is shown. (A) Histogram of blood leukocytes obtained from WT(CD45.1) to WT(CD45.2), WT(CD45.1):CD11c-DTRtg(CD45.2) to WT(CD45.2), and CD11c-DTRtg(CD45.2) to WT(CD45.2) chimera to demonstrate the efficiency of donor bone marrow replenishment and the mixed chimerism of the WT(CD45.1): CD11c-DTRtg(CD45.2) to WT(CD45.2) mice. (B and C) Blood collected from WT(CD45.1) to WT(CD45.2) and WT(CD45.1):DTRtg(CD45.2) to WT(CD45.2) chimera mice that were administered an injection of DT and cisplatin at various time points with respect to cisplatin injection was measured for BUN (B) and serum creatinine (C). $n = 6$ to 7.
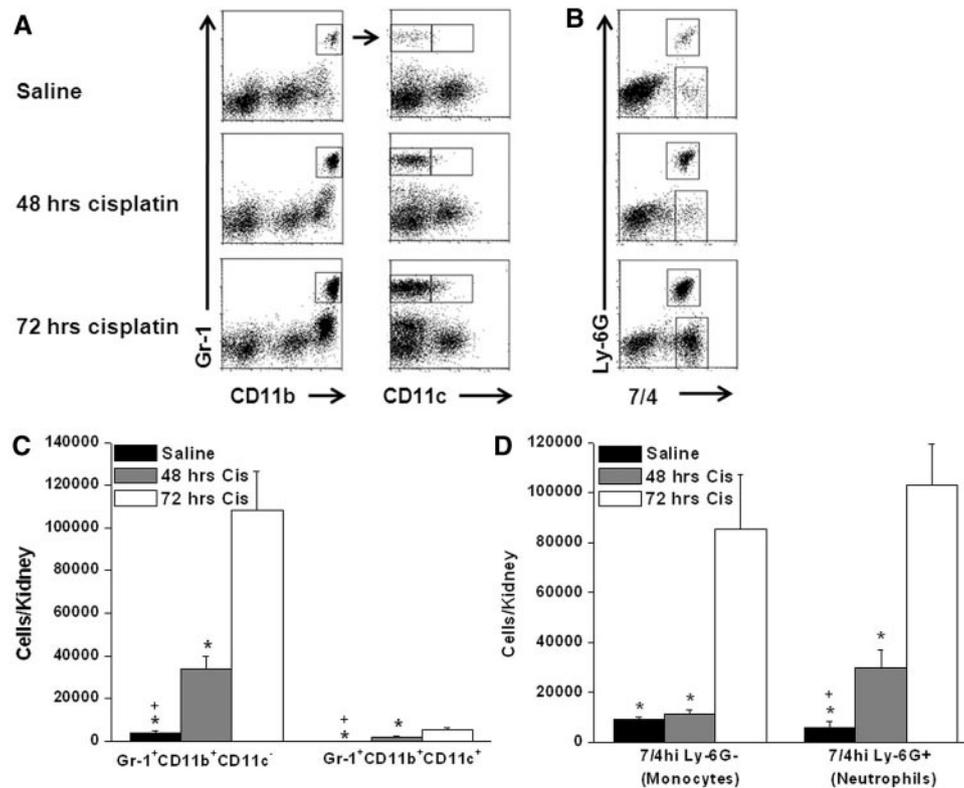
Figure 10. Renal infiltration of inflammatory DCs, neutrophils, and monocytes in cisplatin nephrotoxicity is shown. (A and B) WT mice that were administered an injection of saline or cisplatin were killed at 48 or 72 h, and single-cell renal suspensions were analyzed by flow cytometry gating on CD45$^+$ leukocytes for the expression of Gr-1 *versus* CD11b and CD11c (A) and Ly-6G *versus* 7/4 (B). (C) Absolute number of Gr-1$^+$CD11b$^+$CD11c$^-$ leukocytes and Gr-1$^+$CD11b$^+$CD11c$^+$ inflammatory DCs. (D) Absolute number of 7/4^{hi}Ly-6G$^-$ monocytes and 7/4^{hi}Ly-6G$^+$ neutrophils. *$P < 0.05$ *versus* 72 h cisplatin; $^+P < 0.05$ *versus* 48 and 72 h cisplatin ($n = 3$ to 4).

similar expression of MHCI, CD40, CD80, and CD86. A slight decrease in MHCII expression was noticed in kidney DCs obtained from cisplatin-treated mice. Expression of PDL-1, PDL-2, and FcγR2B were also similar in saline- and cisplatin-treated mice (data not shown). These results indicate that DCs maintained

their steady-state expression of antigen presentation and co-stimulatory molecules in cisplatin-treated mice, at least when examined 24 h after cisplatin treatment. The renal DCs from WT mice treated with saline showed high expression of ICOS-L, which increased significantly in response to cisplatin treatment.
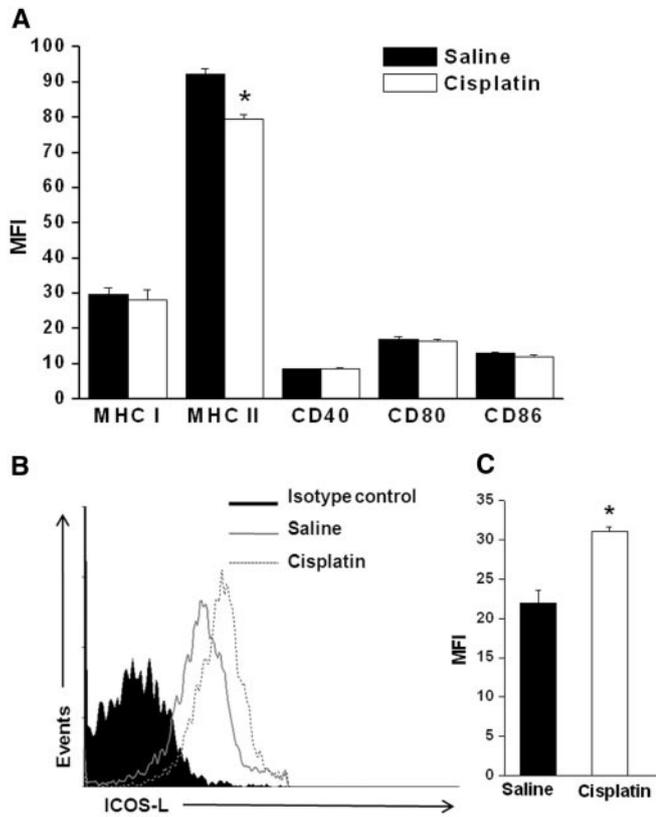
Figure 11. Renal DC expression of surface markers in response to cisplatin treatment is shown. (A through C) WT mice treated with saline or cisplatin were killed at 24 h, and renal DCs (CD45$^+$CD11c$^+$NK1.1$^-$PDCA$^-$) were analyzed for the expression of MHCI, MHCII, CD40, CD80, and CD86 (A) and ICOS-L (B and C) by flow cytometry. *$P < 0.05$ *versus* saline ($n = 3$ to 5).

DISCUSSION

A pathogenic role for inflammation in AKI is well established. Compelling evidence suggests that renal dysfunction in different forms of AKI is the outcome of secretion of immune mediators[5–9] and the activation of renal resident and recruited leukocytes[2,3,10,11]; however, recent studies also suggested that certain cytokines[40–43] and immune cells[23,44,45] may limit kidney injury by reducing the immune-mediated inflammatory response.[46] During renal injury, DCs are thought to contribute to renal inflammation and exacerbate kidney injury.[3,47,48] These studies were based on measures of DC infiltration and cytokine production but did not determine the functional relevance of DCs within their physiologic context *in vivo*. Contrary to these findings, our work used a conditional DC ablation model to demonstrate that resident renal DCs mediate protection in a nephrotoxic model of AKI.

We found an abundant population of CD11c$^+$ cells in the kidney, accounting for almost half of all renal leukocytes. As noted by others,[29,49,50] renal DCs differed from splenic DCs with respect to certain surface markers expression, notably, a high expression of F4/80, a marker of macrophages. Using

CX3CR1 as a marker, Soos *et al.*[29] and Li *et al.*[50] showed DCs in glomeruli and an intricate network throughout the tubulointerstitium. Using CD11c-DTRtg mice, we also observed a network of DCs in the tubulointerstitium but not in glomeruli. This is consistent with a recent report on human kidney.[48] We suspect the glomerular cells seen in the CX3CR1-GFP mice may represent other CX3CR1-expressing cells, such as NK cells, monocytes, or T cells.[51–53]

Although a number of studies have documented changes in the numbers, trafficking, or maturation state of renal DCs in various pathophysiologic conditions,[47,54,55] the role of these renal DCs in their physiologic context remains poorly defined. The functional role of DCs in AKI has been examined using liposomal clodronate.[3,56] In ischemic AKI, clodronate reduced the severity of renal injury,[31,57] whereas in the cisplatin model, clodronate did not affect renal injury.[56] Unfortunately, clodronate is not specific for DCs, which limits the interpretation of these results. In this study, we used a transgenic mouse model to deplete DCs but not macrophages in the kidney. DC ablation before cisplatin treatment caused a marked increase in the severity of cisplatin-induced renal dysfunction, structural damage, and neutrophil infiltration. Through the use of chimeric mice, we determined that the exacerbation of cisplatin-induced AKI was referable to DT effects on hematopoietic cells rather than ectopic expression of the DTR in other sites. These results are consistent with the view that resident renal DCs play a protective role against cisplatin nephrotoxicity. We also determined that renal DCs, in contrast to DCs in skin,[58] are radiosensitive and that bone marrow transplantation effectively replaces the renal DC population with donor cells. This finding has important implications for the design and interpretation of bone marrow chimera studies, which are being increasingly applied to the study of kidney disease.[4,34,59]

During tissue injury, inflammatory DCs may be recruited to the sites of inflammation.[12,27,28] In addition, danger signals released from dying cells may stimulate DCs[37,60,61]; however, we found that depletion of DCs 24 h after cisplatin treatment did not ameliorate cisplatin toxicity and that few inflammatory DCs could be identified. Taken together, these results suggest that proinflammatory DCs are not mediators of cisplatin nephrotoxicity.

The mechanism by which DC ablation exacerbated cisplatin AKI is not certain. Products released from dying cells can stimulate Toll like receptors and induce inflammation.[37,60,61] This is particularly relevant in light of studies from several laboratories,[59,62] including our own,[34] that have identified a role for Toll like receptor signaling in AKI; however, using a novel mixed chimera approach, we were able to demonstrate that dead DCs themselves were not responsible for the exacerbation of AKI. The mixed chimera experiments also indicated that partial (approximately 50%) depletion of DCs did not exacerbate cisplatin AKI. Apparently, the remaining DCs were sufficient for protection.

We also examined the activation state of renal DCs after cisplatin injection. Cisplatin treatment did not result in any difference

in the expression of MHCI, CD40, CD80, and CD86 but caused a slight decrease in the expression of MHCII. Interestingly, renal DCs were found to express high levels of ICOS-L, which increased further after cisplatin treatment. An increase in ICOS-L expression on renal DCs was also observed in DC-mediated amelioration of nephrotoxic nephritis.[23] The findings that renal DCs maintain their steady-state status with respect to the expression of antigen presentation and co-stimulatory molecules and upregulate ICOS-L in response to cisplatin treatment suggests that the modulation of the inflammatory response by DCs may contribute to tissue protection in cisplatin nephrotoxicity.

It is possible that production of IL-10[16] or other anti-inflammatory factors,[13–15] either by DCs themselves or by cells under the influence of DCs, may be an endogenous protective mechanism in AKI. For example, ICOS-L stimulates IL-10 production by T cells,[20–23] and IL-10 has been shown to ameliorate cisplatin nephrotoxicity.[40] DCs might also induce tolerance by capturing apoptotic renal epithelial cells[63] or limit inflammation by ingesting dead renal epithelial cells. In this regard, the phagocytic activity of steady-state immature tissue DCs is high compared with activated mature DCs.[64,65] Resident DCs may also modulate the infiltration of the kidney by other inflammatory cells. In endotoxin-induced uveitis, IL-10 production by DCs may inhibit neutrophil chemotaxis.[66] We observed a greater infiltration of neutrophils into the kidney in DC-depleted mice. Whether this is a cause of or response to the greater kidney injury is not clear from this work. Finally, it should be recognized that DT causes global depletion of DCs in the CD11c-DTRtg mice; therefore, we cannot exclude the possibility that the exacerbation of cisplatin AKI was due to the depletion of extrarenal DCs rather than or in addition to resident renal DCs.

In summary, we have determined the features and distribution of DCs within the kidney and examined their role in a model of toxin-induced AKI. DCs are protective against cisplatin nephrotoxicity. Conditional depletion of DCs accelerates and exacerbates the course of cisplatin-induced AKI. Elucidation of the mechanism by which DCs reduce cisplatin-induced AKI may reveal opportunities for pharmacologic or cell-based interventions. Studies of DCs in other forms of AKI are also warranted.

CONCISE METHODS

Animals

Experiments were performed using 6- to 14-wk-old C57BL6 (CD45.2[+]) or congenic C57BL6 mice B6.SJL-PtprcaPep3b/BoyJ (CD45.1[+]) 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. Bone marrow chimeras were generated as reported previously.[4,34] Acute renal failure was induced in mice by injection of a single dose of cisplatin (20 mg/kg body wt), intraperitoneally. Animals were maintained under specific pathogen-free conditions, and experimental protocols were approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University College of Medicine.

Diphtheria Toxin–Mediated DC Ablation

DCs were depleted in CD11c-DTRtg mice by a single intraperitoneal injection of 4 ng DT/g body weight (Sigma Aldrich). Ablation of DC was confirmed by staining for CD11c^{+} MHCII^{+} DCs in spleen and kidney after perfusion of 20 ml of saline through the heart of the mice. CD11c-DTRtg littermates negative for the transgene were used as controls. Cisplatin was injected into CD11c-DTRtg mice at various times before and after DT injection. In WT mice reconstituted with CD11c-DTRtg bone marrow, 4 ng/g body weight DT was injected twice, 24 h before and 24 h after cisplatin injection.

Renal Function

Renal function was determined by measurement of BUN (VITROS DT60II chemistry slides; Ortho-Clinical Diagnostics, Rochester, NY) and serum creatinine (DZ072B; Diazyme Labs, Poway, CA).

Histologic Examination

Kidneys were fixed in buffered formalin for 24 h, embedded in paraffin, sectioned (4-μm thickness), and stained with periodic acid-Schiff. The extent of tubular injury was assessed using a semiquantitative scale as described previously.[10]

Immunostaining

Formalin-fixed and paraffin-embedded kidney tissue sections of 4-μm thickness were deparaffinized, and antigen retrieval was performed using 10 mM sodium citrate buffer. Immunohistochemistry for neutrophils was performed using a rat anti-mouse neutrophil primary antibody (MCA 771 GA; Serotec) followed by biotinylated anti-rat secondary antibody. For confocal analysis, saline-perfused kidneys were fixed in 2% paraformaldehyde for 2 h at room temperature immediately after excision followed by incubation in 30% sucrose overnight at 4°C and embedded in OCT. Tissue sections stained with DAPI were imaged using a Leica confocal microscope.

Preparation of Single-Cell Suspensions for Flow Cytometry

Mice were subjected to perfusion with 20 ml of saline to remove intravascular leukocytes. Spleen and kidneys were minced into fragments of 1 mm³ and digested with 2 mg/ml collagenase D and 100 U/ml DNase I. The digested tissues were then passed sequentially through 100- and 40-μm mesh. The cell suspension was centrifuged, and red blood cells in the resulting pellet were lysed using red blood cell lysis buffer (Sigma).

Splenic and renal cells were stained using the following fluorochrome-labeled antibodies: Anti-CD45, CD45.1, CD11c, F4/80, MHCI, MHCII, CD11b, CD40, CD80, CD86, Gr-1, Ly-6G (BioLegend), 7/4 (AbD Serotec) CD4, CD8, B220, NK1.1, CD3, ICOS-L, and PDCA-1. Fc receptors were blocked with rat anti-FcR from 2.4G2 hybridoma supernatant. Unless otherwise indicated, the antibodies were obtained from eBioscience or PharMingen. Flow cytometry was performed on FACSCaliber and analyzed using CellQuest (BD PharMingen) or WinMDI 2.8 software (http://facs.scripps.edu/software.html).

Statistical Analysis

Results were expressed as means ± SEM. All data were analyzed using unpaired, two-tailed t test. $P < 0.05$ was considered significant.

ACKNOWLEDGMENTS

DISCLOSURES

None.

REFERENCES

1. Ramesh G, Reeves WB: TNFR2-mediated apoptosis and necrosis in cisplatin-induced acute renal failure. *Am J Physiol Renal Physiol* 285: 610–618, 2003
2. Li L, Huang L, Sung SS, Lobo PI, Brown MG, Gregg RK, Engelhard VH, Okusa MD: NKT cell activation mediates neutrophil IFN-gamma production and renal ischemia-reperfusion injury. *J Immunol* 178: 5899–5911, 2007
3. Dong X, Swaminathan S, Bachman LA, Croatt AJ, Nath KA, Griffin MD: Resident dendritic cells are the predominant TNF-secreting cell in early renal ischemia-reperfusion injury. *Kidney Int* 71: 619–628, 2007
4. Zhang B, Ramesh G, Norbury CC, Reeves WB: Cisplatin-induced nephrotoxicity is mediated by tumor necrosis factor-alpha produced by renal parenchymal cells. *Kidney Int* 72: 37–44, 2007
5. Ramesh G, Reeves WB: TNF-alpha mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity. *J Clin Invest* 110: 835–842, 2002
6. Kielar ML, John R, Bennett M, Richardson JA, Shelton JM, Chen L, Jeyarajah DR, Zhou XJ, Zhou H, Chiquett B, Nagami GT, Lu CY: Maladaptive role of IL-6 in ischemic acute renal failure. *J Am Soc Nephrol* 16: 3315–3325, 2005
7. Furuichi K, Wada T, Iwata Y, Kitagawa K, Kobayashi K, Hashimoto H, Ishiwata Y, Tomosugi N, Mukaida N, Matsushima K, Egashira K, Yokoyama H: Gene therapy expressing amino-terminal truncated monocyte chemoattractant protein-1 prevents renal ischemia-reperfusion injury. *J Am Soc Nephrol* 14: 1066–1071, 2003
8. Miura M, Fu X, Zhang QW, Remick DG, Fairchild RL: Neutralization of Gro alpha and macrophage inflammatory protein-2 attenuates renal ischemia/reperfusion injury. *Am J Pathol* 159: 2137–2145, 2001
9. Melnikov VY, Ecder T, Fantuzzi G, Siegmund B, Lucia MS, Dinarello CA, Schrier RW, Edelstein CL: Impaired IL-18 processing protects caspase-1-deficient mice from ischemic acute renal failure. *J Clin Invest* 107: 1145–1152, 2001
10. Liu M, Chien CC, Burne-Taney M, Molls RR, Racusen LC, Colvin RB, Rabb H: A pathophysiologic role for T lymphocytes in murine acute cisplatin nephrotoxicity. *J Am Soc Nephrol* 17: 765–774, 2006
11. Ascon DB, Lopez-Briones S, Liu M, Ascon M, Savransky V, Colvin RB, Soloski MJ, Rabb H: Phenotypic and functional characterization of kidney-infiltrating lymphocytes in renal ischemia reperfusion injury. *J Immunol* 177: 3380–3387, 2006
12. Heymann F, Meyer-Schwesinger C, Hamilton-Williams EE, Hammerich L, Panzer U, Kaden S, Quaggin SE, Floege J, Grone HJ, Kurts C: Kidney dendritic cell activation is required for progression of renal disease in a mouse model of glomerular injury. *J Clin Invest* 119: 1286–1297, 2009
13. Laouar Y, Town T, Jeng D, Tran E, Wan Y, Kuchroo VK, Flavell RA: TGF-beta signaling in dendritic cells is a prerequisite for the control of autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A* 105: 10865–10870, 2008
14. Munn DH, Sharma MD, Lee JR, Jhaver KG, Johnson TS, Keskin DB, Marshall B, Chandler P, Antonia SJ, Burgess R, Slingluff CL Jr, Mellor AL: Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. *Science* 297: 1867–1870, 2002
15. Travis MA, Reizis B, Melton AC, Masteller E, Tang Q, Proctor JM, Wang Y, Bernstein X, Huang X, Reichardt LF, Bluestone JA, Sheppard D: Loss of integrin alpha (v) beta8 on dendritic cells causes autoimmunity and colitis in mice. *Nature* 449: 361–365, 2007
16. Akbari O, DeKruyff RH, Umetsu DT: Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. *Nat Immunol* 2: 725–731, 2001
17. Desai DD, Harbers SO, Flores M, Colonna L, Downie MP, Bergtold A, Jung S, Clynes R: Fc gamma receptor IIB on dendritic cells enforces peripheral tolerance by inhibiting effector T cell responses. *J Immunol* 178: 6217–6226, 2007
18. Brown JA, Dorfman DM, Ma FR, Sullivan EL, Munoz O, Wood CR, Greenfield EA, Freeman GJ: Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production. *J Immunol* 170: 1257–1266, 2003
19. Hawiger D, Inaba K, Dorsett Y, Guo M, Mahnke K, Rivera M, Ravetch JV, Steinman RM, Nussenzweig MC: Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions *in vivo*. *J Exp Med* 194: 769–779, 2001
20. Akbari O, Freeman GJ, Meyer EH, Greenfield EA, Chang TT, Sharpe AH, Berry G, DeKruyff RH, Umetsu DT: Antigen-specific regulatory T cells develop *via* the ICOS-ICOS-ligand pathway and inhibit allergen-induced airway hyperreactivity. *Nat Med* 8: 1024–1032, 2002
21. Lohning M, Hutloff A, Kallinich T, Mages HW, Bonhagen K, Radbruch A, Hamelmann E, Kroczek RA: Expression of ICOS *in vivo* defines CD4+ effector T cells with high inflammatory potential and a strong bias for secretion of interleukin 10. *J Exp Med* 197: 181–193, 2003
22. Mahnke K, Qian Y, Knop J, Enk AH: Induction of CD4+/CD25+ regulatory T cells by targeting of antigens to immature dendritic cells. *Blood* 101: 4862–4869, 2003
23. Scholz J, Lukacs-Kornek V, Engel DR, Specht S, Kiss E, Eitner F, Floege J, Groene HJ, Kurts C: Renal dendritic cells stimulate IL-10 production and attenuate nephrotoxic nephritis. *J Am Soc Nephrol* 19: 527–537, 2008
24. Jung S, Unutmaz D, Wong P, Sano G, De los Santos K, Sparwasser T, Wu S, Vuthoori S, Ko K, Zavala F, Pamer EG, Littman DR, Lang RA: *In vivo* depletion of CD11c(+) dendritic cells abrogates priming of CD8(+) T cells by exogenous cell-associated antigens. *Immunity* 17: 211–220, 2002
25. van Rijt LS, Jung S, Kleinjan A, Vos N, Willart M, Duez C, Hoogsteden HC, Lambrecht BN: *In vivo* depletion of lung CD11c+ dendritic cells during allergen challenge abrogates the characteristic features of asthma. *J Exp Med* 201: 981–991, 2005
26. Geissmann F, Auffray C, Palframan R, Wirrig C, Ciocca A, Campisi L, Narni-Mancinelli E, Lauvau G: Blood monocytes: Distinct subsets, how they relate to dendritic cells, and their possible roles in the regulation of T-cell responses. *Immunol Cell Biol* 86: 398–408, 2008
27. Serbina NV, Pamer EG: Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nat Immunol* 7: 311–317, 2006

28. Shortman K, Naik SH: Steady-state and inflammatory dendritic-cell development. *Nat Rev Immunol* 7: 19–30, 2007

29. Soos TJ, Sims TN, Barisoni L, Lin K, Littman DR, Dustin ML, Nelson PJ: CX3CR1+ interstitial dendritic cells form a contiguous network throughout the entire kidney. *Kidney Int* 70: 591–596, 2006

30. Dong X, Bachman LA, Miller MN, Nath KA, Griffin MD: Dendritic cells facilitate accumulation of IL-17 T cells in the kidney following acute renal obstruction. *Kidney Int* 74: 1294–1309, 2008

31. Jo SK, Sung SA, Cho WY, Go KJ, Kim HK: Macrophages contribute to the initiation of ischaemic acute renal failure in rats. *Nephrol Dial Transplant* 21: 1231–1239, 2006

32. Bennett CL, Clausen BE: DC ablation in mice: Promises, pitfalls, and challenges. *Trends Immunol* 28: 525–531, 2007

33. Sapoznikov A, Jung S: Probing *in vivo* dendritic cell functions by conditional cell ablation. *Immunol Cell Biol* 86: 409–415, 2008

34. Zhang B, Ramesh G, Uematsu S, Akira S, Reeves WB: TLR4 signaling mediates inflammation and tissue injury in nephrotoxicity. *J Am Soc Nephrol* 19: 923–932, 2008

35. Sapoznikov A, Pewzner-Jung Y, Kalchenko V, Krauthgamer R, Shachar I, Jung S: Perivascular clusters of dendritic cells provide critical survival signals to B cells in bone marrow niches. *Nat Immunol* 9: 388–395, 2008

36. Zammit DJ, Cauley LS, Pham QM, Lefrancois L: Dendritic cells maximize the memory CD8 T cell response to infection. *Immunity* 22: 561–570, 2005

37. Gallucci S, Lolkema M, Matzinger P: Natural adjuvants: Endogenous activators of dendritic cells. *Nat Med* 5: 1249–1255, 1999

38. Fleming TJ, Fleming ML, Malek TR: Selective expression of Ly-6G on myeloid lineage cells in mouse bone marrow: RB6-8C5 mAb to granulocyte-differentiation antigen (Gr-1) detects members of the Ly-6 family. *J Immunol* 151: 2399–2408, 1993

39. Tsou CL, Peters W, Si Y, Slaymaker S, Aslanian AM, Weisberg SP, Mack M, Charo IF: Critical roles for CCR2 and MCP-3 in monocyte mobilization from bone marrow and recruitment to inflammatory sites. *J Clin Invest* 117: 902–909, 2007

40. Deng J, Kohda Y, Chiao H, Wang Y, Hu X, Hewitt SM, Miyaji T, McLeroy P, Nibhanupudy B, Li S, Star RA: Interleukin-10 inhibits ischemic and cisplatin-induced acute renal injury. *Kidney Int* 60: 2118–2128, 2001

41. Kitching AR, Katerelos M, Mudge SJ, Tipping PG, Power DA, Holdsworth SR: Interleukin-10 inhibits experimental mesangial proliferative glomerulonephritis. *Clin Exp Immunol* 128: 36–43, 2002

42. Yin Z, Bahtiyar G, Zhang N, Liu L, Zhu P, Robert ME, McNiff J, Madaio MP, Craft J: IL-10 regulates murine lupus. *J Immunol* 169: 2148–2155, 2002

43. Kluth DC, Ainslie CV, Pearce WP, Finlay S, Clarke D, Anegon I, Rees AJ: Macrophages transfected with adenovirus to express IL-4 reduce inflammation in experimental glomerulonephritis. *J Immunol* 166: 4728–4736, 2001

44. Kanamaru Y, Scandiuzzi L, Essig M, Brochetta C, Guerin-Marchand C, Tomino Y, Monteiro RC, Peuchmaur M, Blank U: Mast cell-mediated remodeling and fibrinolytic activity protect against fatal glomerulonephritis. *J Immunol* 176: 5607–5615, 2006

45. Mahajan D, Wang Y, Qin X, Wang Y, Zheng G, Wang YM, Alexander SI, Harris DC: CD4+CD25+ regulatory T cells protect against injury in an innate murine model of chronic kidney disease. *J Am Soc Nephrol* 17: 2731–2741, 2006

46. Lee VW, Wang YM, Wang YP, Zheng D, Polhill T, Cao Q, Wu H, Alexander IE, Alexander SI, Harris DC: Regulatory immune cells in kidney disease. *Am J Physiol Renal Physiol* 295: 335–342, 2008

47. Fiore N, Castellano G, Blasi A, Capobianco C, Loverre A, Montinaro V, Netti S, Torres D, Manno C, Grandaliano G, Ranieri E, Schena FP, Gesualdo L: Immature myeloid and plasmacytoid dendritic cells infiltrate renal tubulointerstitium in patients with lupus nephritis. *Mol Immunol* 45: 259–265, 2008

48. Segerer S, Heller F, Lindenmeyer MT, Schmid H, Cohen CD, Draganovici D, Mandelbaum J, Nelson PJ, Grone HJ, Grone EF, Figel AM, Nossner E, Schlondorff D: Compartment specific expression of dendritic cell markers in human glomerulonephritis. *Kidney Int* 74: 37–46, 2008

49. Kruger T, Benke D, Eitner F, Lang A, Wirtz M, Hamilton-Williams EE, Engel D, Giese B, Muller-Newen G, Floege J, Kurts C: Identification and functional characterization of dendritic cells in the healthy murine kidney and in experimental glomerulonephritis. *J Am Soc Nephrol* 15: 613–621, 2004

50. Li L, Huang L, Sung SS, Vergis AL, Rosin DL, Rose CE Jr, Lobo PI, Okusa MD: The chemokine receptors CCR2 and CX3CR1 mediate monocyte/macrophage trafficking in kidney ischemia-reperfusion injury. *Kidney Int* 74: 1526–1537, 2008

51. Fong AM, Robinson LA, Steeber DA, Tedder TF, Yoshie O, Imai T, Patel DD: Fractalkine and CX3CR1 mediate a novel mechanism of leukocyte capture, firm adhesion, and activation under physiologic flow. *J Exp Med* 188: 1413–1419, 1998

52. Imai T, Hieshima K, Haskell C, Baba M, Nagira M, Nishimura M, Kakizaki M, Takagi S, Nomiyama H, Schall TJ, Yoshie O: Identification and molecular characterization of fractalkine receptor CX3CR1, which mediates both leukocyte migration and adhesion. *Cell* 91: 521–530, 1997

53. Geissmann F, Jung S, Littman DR: Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity* 19: 71–82, 2003

54. Woltman AM, de Fijter JW, Zuidwijk K, Vlug AG, Bajema IM, van der Kooij SW, van Ham V, van Kooten C: Quantification of dendritic cell subsets in human renal tissue under normal and pathological conditions. *Kidney Int* 71: 1001–1008, 2007

55. Loverre A, Capobianco C, Stallone G, Infante B, Schena A, Ditonno P, Palazzo S, Battaglia M, Crovace A, Castellano G, Ranieri E, Schena FP, Gesualdo L, Grandaliano G: Ischemia-reperfusion injury-induced abnormal dendritic cell traffic in the transplanted kidney with delayed graft function. *Kidney Int* 72: 994–1003, 2007

56. Lu LH, Oh DJ, Dursun B, He Z, Hoke TS, Faubel S, Edelstein CL: Increased macrophage infiltration and fractalkine expression in cisplatin-induced acute renal failure in mice. *J Pharmacol Exp Ther* 324: 111–117, 2008

57. Day YJ, Huang L, Ye H, Linden J, Okusa MD: Renal ischemia-reperfusion injury and adenosine 2A receptor-mediated tissue protection: Role of macrophages. *Am J Physiol Renal Physiol* 288: 722–731, 2005

58. Merad M, Manz MG, Karsunky H, Wagers A, Peters W, Charo I, Weissman IL, Cyster JG, Engleman EG: Langerhans cells renew in the skin throughout life under steady-state conditions. *Nat Immunol* 3: 1135–1141, 2002

59. Wu H, Chen G, Wyburn KR, Yin J, Bertolino P, Eris JM, Alexander SI, Sharland AF, Chadban SJ: TLR4 activation mediates kidney ischemia/reperfusion injury. *J Clin Invest* 117: 2847–2859, 2007

60. Shi Y, Evans JE, Rock KL: Molecular identification of a danger signal that alerts the immune system to dying cells. *Nature* 425: 516–521, 2003

61. Kono H, Rock KL: How dying cells alert the immune system to danger. *Nat Rev Immunol* 8: 279–289, 2008

62. Leemans JC, Stokman G, Claessen N, Rouschop KM, Teske GJ, Kirschning CJ, Akira S, van der Poll T, Weening JJ, Florquin S: Renal-associated TLR2 mediates ischemia/reperfusion injury in the kidney. *J Clin Invest* 115: 2894–2903, 2005

63. Steinman RM, Turley S, Mellman I, Inaba K: The induction of tolerance by dendritic cells that have captured apoptotic cells. *J Exp Med* 191: 411–416, 2000

64. Steinman RM, Hawiger D, Nussenzweig MC: Tolerogenic dendritic cells. *Annu Rev Immunol* 21: 685–711, 2003

65. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, Palucka K: Immunobiology of dendritic cells. *Annu Rev Immunol* 18: 767–811, 2000

66. Mashimo H, Ohguro N, Nomura S, Hashida N, Nakai K, Tano Y: Neutrophil chemotaxis and local expression of interleukin-10 in the tolerance of endotoxin-induced uveitis. *Invest Ophthalmol Vis Sci* 49: 5450–5457, 2008