A Pathophysiologic Role for T Lymphocytes in Murine Acute Cisplatin Nephrotoxicity

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Recent evidence supports a role for an inflammatory pathogenesis of cisplatin nephrotoxicity, but immune cell-mediated mechanisms in this disease are still largely unknown. The role for T lymphocytes on cisplatin-induced acute kidney injury was examined with C57BL/6 T cell–deficient (nu/nu) mice and CD4– or CD8-deficient mice and their wild-type (WT) littermates. All mice received a single dose of cisplatin at 40 mg/kg (intraperitoneally) and were followed up for 72 h. At 72 h after cisplatin administration, T cell–deficient mice had a marked attenuation in renal dysfunction (serum creatinine 3.2 ± 0.5 versus 0.8 ± 0.1 mg/dl; P = 0.007), kidney tubular injury (scores 1.44 ± 0.15 versus 0.22 ± 0.08; P < 0.0001), and survival. Adoptive transfer of T cells into nu/nu mice followed by cisplatin enhanced renal dysfunction and tubular injury. The increase in renal myeloperoxidase activity after cisplatin administration was blunted in nu/nu mice. Renal TNF-α, IL-1β, and keratinocyte-derived chemokine protein expression was increased in WT mice but not in nu/nu mice after cisplatin administration. T cell levels significantly increased in kidneys of WT mice after cisplatin administration as early as at 1 h, peaked at 12 h, and declined by 24 h. CD4- and, to a lesser degree, CD8-deficient mice were relatively protected from cisplatin-induced mortality and renal dysfunction compared with WT mice. These data demonstrate that T lymphocytes are direct mediators of experimental cisplatin nephrotoxicity. Targeting T lymphocytes could lead to improved ways to administrer cisplatin safely to cancer patients.

Cisplatin is an effective chemotherapeutic agent that is widely used in the treatment of a variety of malignancies, including head and neck, ovarian, and testicular cancers. However, nephrotoxicity, the most common adverse effect, limits the use of this drug in many cancer patients (1). Approximately 25 to 30% of patients developed renal dysfunction after receiving a single dose of cisplatin (2). The pathogenesis of cisplatin toxicity is attributed to the formation of reactive oxygen species (3), caspase activation (4), DNA damage (5,6), and mitochondrial damage (7). Apoptosis, necrosis, and inflammation have also been recognized as important mechanisms of cisplatin nephrotoxicity in vivo and in vitro (8,9).

Recent studies have shown that cisplatin upregulates the expression of TNF-α in mouse kidney, and the level of TNF-α correlates with the severity of renal injury (10,11). Furthermore, inhibiting TNF-α release or its activity with an antagonist/inactive analogue or using mice with specific genetic defects in TNF-α receptor 2 protects mice from cisplatin-induced kidney injury (12,13). Moreover, T cells have been shown to be important modulators of ischemia reperfusion injury (IRI) in the kidney, liver, lung, and intestine (14–17). Given that T cells have been shown to mediate ischemic acute kidney injury (AKI), along with recent data supporting an inflammatory component to cisplatin toxicity, we hypothesized that T cells could mediate cisplatin-induced AKI.

In this study, we compared the nephrotoxic response to cisplatin in T cell–deficient (nu/nu) mice and CD4–/– or CD8–/– mice with their wild-type (WT) controls. We found that nu/nu mice had marked improvement in survival, renal dysfunction, and histologic injury when compared with the WT mice that were exposed to cisplatin. Adoptive transfer of T cells into nu/nu mice increased cisplatin nephrotoxicity. T cells infiltrated into the WT kidneys after cisplatin administration within 1 h, peaked at 12 h, and declined by 24 h. The increase in renal myeloperoxidase (MPO) activity in WT mice at 72 h after cisplatin administration was significantly attenuated in nu/nu mice. In addition, proinflammatory mediators, including TNF-α, IL-1β, and keratinocyte-derived chemokine (KC), increased in WT mice kidney 72 h after cisplatin administration; however, in nu/nu mice, the increase was attenuated. CD4 deficiency and, to a lesser degree, CD8 deficiency also conferred protection from cisplatin-induced nephrotoxicity.

Materials and Methods

Animals

All animal study protocols were reviewed and approved by the Animal Care and Use Committee of Johns Hopkins University, and all experiments were conducted according to National Institutes of Health guidelines. T cell–deficient athymic male mice (B6.Cg-Foxn1nu, nu/nu)
and their C57BL/6 WT male littermates (6 to 8 wk of age, weighing 20 to 25 g) were purchased from The Jackson Laboratory (Bar Harbor, ME). The two main defects of T cell–deficient mice homozygous for the nu/nu spontaneous mutation (Foxn1nu, formerly Hbb11nu) are the abnormal hair growth and defective development of the thymus. Consequently, homozygous nu/nu mice lack T cells and cell-mediated immunity. Strain-matched WT male littermates were used as controls and as donors of T cell adoptive transfer. CD4-deficient mice (B6.129S2-Cd4tm1Mak) and CD8-deficient mice (B6.129S2-Cd8atm1Mak) and their WT littermates were also purchased from The Jackson Laboratory. Mice were kept under pathogen-free conditions in Johns Hopkins Medical Institutions animal facility with air conditioning and a 14-h/10-h light/dark cycle. All mice had free access to food and water during the experiments.

Cisplatin Administration and Tissue Collection

Cisplatin (cis-diammineplatinum II dichloride; Sigma-Aldrich, St. Louis, MO) was dissolved in 0.9% of saline at a concentration of 1 mg/ml. Mice were given a single intraperitoneal injection either of cisplatin (40 mg/kg body wt) or of equal volume of saline. This dose was chosen on the basis of our preliminary studies that lower doses did not give consistent and significant renal dysfunction and tubular injury, but a dose of 40 mg/kg produced a predictable combination of survivability and AKI from as early as 24 h and reached a peak at 72 h after cisplatin administration in C57BL/6 WT mice. For kidney T cell immunohistochemical staining, three WT mice in each group were sacrificed at 1, 6, 12, 24, and 72 h after cisplatin injection. Mice also were sacrificed at 24 and 72 h after the cisplatin administration for histology and kidney cytokine array analysis. All collected mouse kidneys were either fixed in 10% buffered formalin solution and then analyzed by FACSCalibur (Becton Dickinson Immunocytometry Systems, San Jose, CA) using a Creatinine 557 kit (Sigma Diagnostics, St. Louis, MO) to visualize the immunocomplex and counterstained with hematoxylin. All cisplatin kidney sections were examined by a pathologist and a nephrologist in a blinded manner, and CD3-positive cells were counted in at least 10 corticomedullary fields.

Assessment of Renal Function

Blood samples were obtained from mice before (0 h) and at 24, 48, and 72 h after cisplatin injection. Serum creatinine was measured as a marker of renal dysfunction by a Roche Cobas Fara automated system (Roche, Nutley, NJ) using a Creatinine 557 kit (Sigma Diagnostics, St. Louis, MO). Blood samples were obtained from mice before (0 h) and at 24, 48, and 72 h after cisplatin injection. Serum creatinine was measured as a marker of renal dysfunction by a Roche Cobas Fara automated system (Roche, Nutley, NJ) using a Creatinine 557 kit (Sigma Diagnostics, St. Louis, MO). Serum creatinine was measured as a marker of renal dysfunction by a Roche Cobas Fara automated system (Roche, Nutley, NJ) using a Creatinine 557 kit (Sigma Diagnostics, St. Louis, MO).

Histologic Examination

Formalin-fixed and paraffin-embedded sections of mouse kidney tissues were cut and stained with hematoxylin and eosin. Renal tubular injury was assessed using a semiquantitative scale. A pathologist who was blinded to the experiments scored the degree of tubular injury. The magnitude of tubular epithelial cell loss, necrosis, intratubular debris, and tubular cast formation was scored into six levels on the basis of the percentage of affected tubules in a high-power field under light microscope: 0, none; 0.5, <10%; 1, 10 to 25%; 2, 25 to 50%; 3, 50 to 75%; and 4, >75%.

T Cell Adoptive Transfer

One group of nu/nu mice (n = 5) received an adoptive transfer of T cells from WT mice. Briefly, spleens that were collected from C57BL/6 WT littermates were minced on a nylon mesh and filtered through a cell strainer (70 μm). The obtained cell suspension underwent red blood cell lysis using a red blood cell lysis buffer (eBioscience, San Diego, CA). T cells were enriched by mouse T cell enrichment columns (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions. After enrichment treatment, the purity of the T cell suspension was >90%. Approximately 3 × 10⁶ enriched T cells were injected intraperitoneally into each nu/nu mouse 3 wk before cisplatin administration. This interval was chosen on the basis of our preliminary studies that showed that earlier time points had less efficient reconstitution (14).

FACS Analysis of Splenic T Cell Reconstitution

To confirm T cell reconstitution in nu/nu mice, spleens were collected from nu/nu mice with or without T cell transfer and from their WT littermates upon being killed at 72 h after cisplatin administration. Splenocytes were isolated and analyzed for a population of CD3-positive cells (pan T cells) by flow cytometry. Briefly, isolated splenocytes were blocked with an Fcγ III/II receptor and stained directly with a FITC-conjugated anti-mouse CD3 (17A2) mAb (BD Pharmingen, San Diego, CA) for 20 min at room temperature. The stained cells then were fixed in 1% of formalin solution and then analyzed by FACS caliber using Cell Quest software V3.3 (Becton Dickinson Immunocytometry Systems, San Jose, CA). The T cell population was expressed as a percentage of gated CD3-positive cells of total splenocytes.

Immunohistochemical Staining of T Cells

To evaluate T cell infiltration into kidneys after cisplatin administration as a potential mechanism of action, immunohistochemical staining for T cells was performed on formalin-fixed kidney tissue. Briefly, after deparaffinization and rehydration, kidney sections were immersed in 3% of hydrogen peroxide methanol for 5 min to block endogenous peroxidase. For antigen retrieval, slides were pressure-cooked in Antigen-decloaker solution (Biocare Medical, Walnut Creek, CA) for 3 min. After treatments with normal goat serum (1:100) and two drops of avidin D (100 mg/ml PBS), a polyclonal rabbit anti-human/mouse CD3 antibody was added at a 1:200 dilution (Calbiochem, San Diego, CA) and incubated overnight at 4°C. This was followed by an incubation with a biotinylated goat anti-rabbit IgG for 35 min and with Streptavidin Peroxidase (Biogenex, San Ramon, CA) for 45 min. Finally, the kidney sections were exposed with Romulin AEC Chromogen (Biocare Medical) to visualize the immunocomplex and counterstained with hematoxylin. All cisplatin kidney sections were examined by a pathologist and a nephrologist in a blinded manner, and CD3-positive cells were counted in at least 10 corticomedullary fields.

Kidney MPO Assay

Renal MPO activity was measured as described by Laight et al. (18) in nu/nu mice and WT mice at 72 h after receiving cisplatin to semiquantify neutrophil and macrophage infiltration. Briefly, kidney tissue was homogenized in a solution that contained 0.5% (wt/vol) hexadecyltrimethylammonium bromide dissolved in 50 mM potassium phosphate buffer (pH 6.0) and centrifuged for 30 min at 20,000 × g at 4°C. Samples were incubated in a water bath at 60°C for 2 h and then centrifuged at 4000 × g for 12 min. The collected supernatant (40 μl) in each sample was incubated with 160 μl of a reaction solution that contained 1.6 mM tetrathylbenzidine and 3 mM H₂O₂ diluted in 80 mM phosphate buffer (pH 5.4) in a 96-well microplate. The rate of change in absorbance at 630 nm over 5 min was measured spectrophotometrically. MPO activity was expressed as absorbance changed per minute per 100 mg of wet tissue.

Kidney Cytokine Protein Array

To examine proinflammatory molecules that were generated by cisplatin injury, protein levels of IL-1β, IFN-γ, TNF-α, and KC were measured in mouse kidneys using a Bio-Plex multiple cytokines array technique (Bio-Rad Laboratories Inc., Hercules, CA) previously described in depth (19) with a simplified small template that contained only the above four cytokine/chemokines. Briefly, snap-frozen kidney
tissue was homogenized in a cell lysis buffer, and the homogenates were centrifuged at 12,000 rpm for 15 min at 4°C. Total protein concentration in each supernatant was determined using a Bio-Rad Protein Assay Kit, and the measured protein level in each sample was adjusted to 500 μg/ml with cell lysis buffer. Each sample first was incubated with a mixture of all types of microbeads for 90 min at room temperature followed by an incubation with biotinylated detection antibodies for 30 min, then with a streptavidin-coupled phycoerythrin for 10 min (room temperature). Finally, the samples were subjected to a flow cytometric system. All acquired data were analyzed using Bio-Plex Manager 3.0 software (Bio-Rad) and corrected for total protein concentration (pg/mg protein).

Statistical Analyses

Data are expressed as mean ± SEM and are compared by an unpaired, two-tailed t test for single comparison or by an ANOVA post hoc test for multiple comparison. Kaplan-Meier analysis was used for mouse survival analyses. Statistical significance of difference was defined as P < 0.05.

Results

T Cell–Deficient Mice Had Marked Improved Survival after Cisplatin Administration

Both T cell–deficient (nu/nu) mice and their C57BL/6 WT littermates received a single dose of cisplatin at 40 mg/kg and were followed up to 3 d. At 72 h after injection of cisplatin, only eight of 14 WT mice survived (58% of survival rate). Meanwhile, nu/nu mice had 100% survival, with all 12 mice alive at 72 h after cisplatin administration (Figure 1).

T Cell–Deficient Mice Had Marked Protection from Cisplatin-Induced Renal Dysfunction

Cisplatin administration led to the development of AKI with a rise in serum creatinine from 0.7 (baseline) to 3.61 mg/dl by 72 h after cisplatin administration in WT mice. In contrast, nu/nu mice that received cisplatin had significant attenuation in serum creatinine elevation at 24 (1.05 ± 0.11 versus 0.60 ± 0.05; P < 0.02), 48 (2.09 ± 0.49 versus 0.56 ± 0.05; P < 0.05), and 72 h (3.61 ± 0.32 versus 0.58 ± 0.06; P < 0.0001; Figure 2) when compared with WT mice.

T Cell Deficiency Protects Mice from Renal Tubular Injury Induced by Cisplatin

At 72 h after cisplatin administration, WT mice developed extensive renal tubular injury. However, nu/nu mice had sig-

Figure 1. Survival in cisplatin-treated wild-type (WT) mice and nu/nu mice. All mice received a single dose of cisplatin (intraperitoneally, 40 mg/kg) and were followed up to 72 h. Compared with 58% survival rate in WT mice, nu/nu mice had 100% survival rate at 72 h after cisplatin administration (n = 12 to 14).

Figure 2. Renal function in cisplatin-treated WT mice and nu/nu mice. Serum creatinine was measured before (0 h) and at 24, 48, and 72 h after injection of cisplatin (40 mg/kg). Compared with WT mice, nu/nu mice had significantly reduced creatinine at all time points (*P = 0.01, **P < 0.05, ***P < 0.0001 versus WT; n = 5 to 7).

Figure 3. Renal tubular injury scores in cisplatin-treated WT mice and nu/nu mice. The degree of renal tubular injury at 72 h after cisplatin administration (40 mg/kg) in WT mice and nu/nu mice was scored using an established method of semiquantitative evaluation. Compared with WT mice that developed extensive tubular injury with a high score, the nu/nu mice had significantly less tubular injury (*P < 0.0001).
nificantly less tubular injury (injury scores 1.44 ± 0.15 versus 0.22 ± 0.08; P < 0.0001; Figure 3).

**T Cell Adoptive Transfer Reconstituted Kidney Susceptibility to Cisplatin Toxicity**

To determine whether T cell deficiency in nu/nu mice was indeed the protective factor in cisplatin-induced renal injury, we transferred 3 × 10⁶ purified splenic T cells (purity >90%) from normal WT mice into each of five nu/nu mice. The successful transfer of T cells was confirmed by FACS analysis with CD3 staining. The mean population of T cells in WT mouse spleen was 7.7% of total splenocytes. Meanwhile, nu/nu mice had minimal (0.4%) splenic T cells. Three weeks after transfer, the splenic T cells in nu/nu mice were reconstituted to 2.4%, 35% of those in WT mice (Figure 4).

T cell transfers led to a significant enhancement of renal dysfunction in nu/nu mice. There was a significant rise in serum creatinine in the nu/nu mice that received a transfer of T cells when compared with the nu/nu mice alone (without transfer) at 72 h after cisplatin administration (0.58 ± 0.06 versus 1.23 ± 0.11; P < 0.04; Figure 5). Nu/nu mice with a T cell transfer also developed significant renal tubular injury (Figure 6), confirmed by a semiquantitative scoring of tubular injury (Figure 7).

**T Cells Infiltrate Early into Mouse Kidneys after Cisplatin Administration**

To investigate whether T cells infiltrate into kidneys after cisplatin administration at early time points, three WT mice in each of the five groups were treated with a single dose of cisplatin and were sacrificed at 1, 6, 12, and 24 h after cisplatin injection. Kidney tissues were stained with anti-CD3 antibody, a pan marker for T cell, by immunohistochemistry. There was markedly increased CD3-positive cell staining detected in kidney as early as at 1 h after cisplatin administration. This increase of T cells reached a peak at 12 h and then declined by 24 h (Figures 8 and 9, *P < 0.004, **P < 0.0002).}

*Figure 5. Effect of T cell transfer on post-cisplatin renal function. Serum creatinine was measured in WT mice, nu/nu mice alone, and nu/nu mice with a T cell transfer after a single dose of cisplatin (40 mg/kg). Compared with nu/nu mice alone, there was a significant rise in serum creatinine in the nu/nu mice with a T cell transfer (*P < 0.05 versus nu/nu mice alone; **P = 0.002; ***P < 0.0001 versus WT mice; n = 3 to 5).*

**T Cell Deficiency Attenuates Increased Renal MPO Activity Late after Cisplatin Administration**

To assess renal phagocyte infiltration after cisplatin treatment, we measured MPO activity in kidneys after cisplatin administration in nu/nu mice and WT mice that were treated either with cisplatin or with saline as negative controls. Compared with their individual saline controls, both WT and nu/nu mice had significant increases in renal MPO activity at 72 h after receiving cisplatin. However, this increase was blunted in nu/nu mice when compared with the WT mice after cisplatin administration (Figure 10).

*Figure 4. FACS analysis of mouse splenic CD3-positive T cells. One group of nu/nu mice (n = 5) received a T cell adoptive transfer 3 wk before cisplatin administration. Splenocytes were isolated from each mouse upon killing and stained with FITC-conjugated anti-mouse CD3 antibody and analyzed by FACS. WT mice had (7.7 ± 0.3%) of CD3+ splenic T cells; meanwhile, nu/nu mice had minimal T cells (0.4 ± 0.1%) in their spleen. Three weeks after receiving an adoptive transfer of WT T cells, the average population of splenic T cells was reconstituted up to (2.4 ± 0.4%; nu/nu versus, nu/nu + T cell, P = 0.0005).*
Effect of CD4 or CD8 Deficiency on Cisplatin-Induced Mortality

To dissect the individual roles of the CD4 and CD8 T cell subsets on the outcome of cisplatin-induced renal injury, we compared the effects of cisplatin on mortality in the mice that were deficient in either CD4 or CD8 T cell. These deficient mice as well as their WT littermates received a single dose (40 mg/kg, intraperitoneally) of cisplatin and were followed for up to 72 h. In the CD4 and CD8 WT littermates, despite being on a similar background to the nu/nu mice WT littermates, there seemed to be enhanced susceptibility to cisplatin toxicity at the identical dose and administration regimens. Because most WT mice died by 48 h and few mice survived until 72 h after cisplatin treatment, the total number of mice was significantly increased. By 72 h after cisplatin treatment, only five (20%) of 25 WT mice survived, meanwhile 10 (62%) of 16 CD4-deficient mice and seven (43%) of 16 CD8-deficient mice survived (Figure 11, *P < 0.012 by log-rank test).

Effect of CD4 or CD8 Deficiency on Cisplatin-Induced Renal Dysfunction

WT mice developed a progressive rise in serum creatinine, whereas CD4-deficient mice had significantly reduced creatinine level at 48- and 72-h time points compared with WT mice. CD8-deficient mice were less protected than CD4-deficient mice, with the changes in serum creatinine in CD4-deficient mice compared with WT mice only statistically significant at the 72-h time point (serum creatinine, WT versus CD4−/− versus CD8−/−: 48 h 3.05 ± 0.43 versus 1.27 ± 0.31 versus 2.38 ± 0.63 mg/dl; 72 h 3.53 ± 0.28 versus 1.65 ± 0.36 versus 1.93 ± 0.45 mg/dl; Figure 12). Histologic changes were consistent with the serum creatinines in this group (Figure 13).

T Cell Deficiency Attenuates Renal Cytokine/Chemokine Expression after Cisplatin Administration

To determine potential soluble mediators of T cell in cisplatin-induced nephrotoxicity, we measured protein levels of IL-β, KC, IFN-γ, and TNF-α in mouse kidney at 24 and 72 h after cisplatin injection. Compared with saline controls, cisplatin-treated WT mice had increased levels of renal IL-1β, KC, and TNF-α (all as pg/mg protein) at 72 h after injection of cisplatin (IL-1β 27.91 ± 1.62 versus 53.43 ± 2.61, P = 0.0005; KC 16.87 ±
versus 338.30, \( P = 0.0004 \); TNF-\( \alpha \) 495.21 \( \pm \) 44.05 versus 707.40 \( \pm \) 66.28, \( P < 0.02 \)). However, \( nu/nu \) mice that were treated with cisplatin had a reduced expression of these proinflammatory molecules in the kidney. There was no increase in IFN-\( \gamma \) protein levels in both \( nu/nu \) and WT mice at any time point after cisplatin administration (Figure 14).

### Discussion

Nephrotoxicity is a major limitation for administering adequate doses of the chemotherapeutic agent cisplatin. Most studies support a role for apoptosis/necrosis and reactive oxygen species in the pathogenesis of cisplatin-induced renal injury (3,8). Recently, an inflammatory basis for cisplatin toxicity was demonstrated with a role for intercellular adhesion molecule-1,
TNF-α, and other proinflammatory molecules (10,11,20). Given that inflammatory mechanisms have been found also to participate in the pathogenesis of kidney IRI and T cells can modulate the outcome of kidney IRI (14), we hypothesized that T cells also could mediate cisplatin-induced renal toxicity. Our data demonstrate, for the first time, that T cells directly mediate the pathogenesis of cisplatin-induced acute nephrotoxicity. The marked functional and structural protection seen in T cell–deficient nu/nu mice corresponded with a survival advantage.

To ensure that T cell deficiency in nu/nu mice was directly related to the protective effect, we performed T cell adoptive transfers on nu/nu mice, which significantly restored both structural and functional injury after cisplatin administration. T cells infiltrated into kidneys within hours after cisplatin treatment, demonstrating a potential role for early T cell trafficking in this injury process. Primarily CD4 cells and, to a lesser degree, CD8 T cells play role in cisplatin toxicity—a similar hierarchy to what is observed in ischemic AKI. Infiltration of neutrophils and macrophages into kidney, represented by renal MPO activity late after cisplatin, was attenuated in T cell–deficient mice, indicating a potential effector mechanism. Proinflammatory molecules were also measured in kidney to explore other potential effector mechanisms. After cisplatin treatment, the protein levels of TNF-α, KC, and IL-1β were increased in WT mouse kidneys, and these increases were blunted in nu/nu mouse kidneys.

Although we predicted that T cells could play a role in cisplatin-induced nephrotoxicity, the marked degree of protection from renal functional decline and structural injury in the T cell–deficient nu/nu mouse strain was unexpected. The protec-
tion in the nu/nu mouse from cisplatin was more prominent than the role of the T cell in renal IRI previously described (14,21). Given that the T cell–deficient nu/nu mice could have other abnormalities besides T cell deficiency leading to resistance to cisplatin toxicity, we then studied the effects of adoptive transfer of purified WT splenic T cells into nu/nu mice on cisplatin toxicity. Even by conservative estimates of the degree of reconstitution of splenic T cells in the nu/nu mice, we found significant worsening in kidney function and tubular injury, validated with a semiquantitative scoring in a blinded manner. This is similar to the effect of T cells seen in renal IRI and in allograft models in T cell–deficient mice as described previously (14). Of note, the rise in serum creatinine in the T cell–reconstituted nu/nu mice was not as prominent as the histologic worsening. This is consistent with the notion that major changes in kidney structure do not track identically with serum creatinines. Longer term follow-up of these T cell–reconstituted mice may have shown an even greater rise in serum creatinine with time.

We then began to explore the potential mechanisms by which T cells could mediate cisplatin-induced nephrotoxicity. T cell trafficking into a target organ is an important basis for T cell–mediated injury in many diseases such as transplant rejection (22). Using an anti-CD3 polyclonal antibody specific for T cells in mouse tissue, we found a significant increase in T cell infiltration into mouse kidney within hours of cisplatin administration that decreased by 24 and 72 h. This early trafficking could underlie the effects of T cells in this nephrotoxic model. However, there are also examples in other inflammatory diseases, such as in experimental asthma, for which one can dissociate T cell trafficking from a T cell–mediated functional effect (23). Previous data have demonstrated a significant role for CD4 cells in ischemic acute tissue injury in the kidney (14), liver (15), and lung (16). We therefore hypothesized that CD4 T cells would be the main T cell subset that mediated cisplatin-induced acute renal failure. We found that CD4 deficiency conferred a relative protection in renal function and mortality after cisplatin. Previous work suggests that this could be mediated through the production of deleterious Th1 polarized cytokines from CD4 cells (24). We also found a protection afforded by CD8 deficiency, although this was not as prominent as CD4 deficiency. In contrast, in a murine model of Adriamycin-induced nephrotoxicity, CD4 T cells, traditionally considered as regulator or proinflammatory helper cells, were shown to improve outcome, whereas CD8 T cells promoted renal injury (25,26).

Even though early recruitment of T cells was observed, the small numbers suggested that other potent effector mecha-

Figure 14. Proinflammatory protein array in post-cisplatin mouse kidneys. Kidneys were harvested at 24 or 72 h after cisplatin injection, and a multiplex cytokine/chemokine protein array was performed by Bio-Rad multiplex techniques. Compared with the baseline controls, there was a significant increase in IL-1β, keratinocyte-derived chemokine (KC), and TNF-α in WT cisplatin-treated mice at 72 h. At this time point, the nu/nu mice had a significantly reduced increase in these cytokines when compared with WT cisplatin-injected mice. No significant increase was found in IFN-γ at either 24 or 72 h after cisplatin administration in both WT and nu/nu mice (*P < 0.002, **P < 0.01, ***P < 0.02 versus WT 72 h; n = 3 in each group).
nisms were in play. We therefore measured infiltrating phagocytes (neutrophils and macrophages) using MPO activity assay. Kidney MPO levels were increased in both WT and nu/nu mice after cisplatin; however, this increase was reduced in nu/nu mice. Thus, T cell–mediated phagocyte infiltration is a potential mode of action and is consistent with recent publications on the mechanisms of T cells in ischemic tissue injury (17,27). Given that recent data have demonstrated a significant role for TNF-α in cisplatin nephrotoxicity, we measured several cytokine/chemokine protein expressions in both cisplatin-treated nu/nu and WT mouse kidneys as potential mediators of the T cell’s role in cisplatin nephrotoxicity. We found an increase in TNF-α, IL-1β, and KC at time points in conjunction with the rise in serum creatinine and tubular injury in WT mice. However, these increases were blunted in the nu/nu mice. Thus, these molecules may be potential effectors of T cell–mediated cisplatin toxicity. However, it is also possible that these cytokines could be associated with the decreased injury rather than cause and effect or could be markers for damage and inflammation caused by other agents.

An improved understanding of the pathophysiology of cisplatin nephrotoxicity should lead to improved preventive and therapeutic strategies. Peroxisome proliferator–activated receptor-α ligands can be used also to prevent cisplatin nephrotoxicity (28). This compound has been shown to have a profound inhibitory effect on T cell function by impaired production of TNF-α (29). Novel strategies toward T cell modulation could be effective in reducing toxicity from this effective chemotherapeutic agent.

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