Anaphylatoxin C5a contributes to the pathogenesis of cisplatin-induced nephrotoxicity

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Pan H, Shen Z, Mukhopadhyay P, Wang H, Pacher P, Qin X, Gao B. Anaphylatoxin C5a contributes to the pathogenesis of cisplatin-induced nephrotoxicity. Am J Physiol Renal Physiol 296:F496–F504, 2009. First published January 14, 2009; doi:10.1152/ajprenal.90443.2008.—Nephrotoxicity is a common complication of cisplatin chemotherapy that limits its clinical use; however, the mechanisms underlying cisplatin-induced nephrotoxicity are not fully understood. In this study, we investigated the role of anaphylatoxin C5a in the pathogenesis of cisplatin-mediated nephrotoxicity. Our data show that cisplatin-induced renal injury is significantly reduced in C5- or C5aR-deficient mice. However, pretreatment with C5 or C5a restores sensitivity to cisplatin-induced nephrotoxicity in C5-deficient mice. In wild-type mice, administration of cisplatin triggers the increased renal expression of multiple cytokines and caspases. This induction is diminished in C5-deficient mice, which is restored by pretreatment with C5 or C5a proteins. Interestingly, renal injury induced by cisplatin is similar between wild-type and CD59ab double knockout mice, and the formation of membrane attack complexes (MACs) by cisplatin in the kidney is diminished in C5-deficient mice, but not in C5aR-deficient mice. In conclusion, our findings suggest that C5a plays an important role in the pathogenesis of cisplatin nephrotoxicity. Likely, C5a binds to C5aR, leading to induction of proinflammatory cytokines and inflammation. The formation of MACs does not appear to contribute to the nephrotoxicity of cisplatin based on our study results.

cytokines; caspase; STAT3; NF-κB

A PLATINUM COMPOUND, CISPLATIN, is one of the most potent chemotherapeutic agents available that is widely used to treat a variety of malignancies, including ovarian, lung, head, and neck cancers, as well as testicular and bladder tumors (25). Unfortunately, at high doses, cisplatin induces cumulative and dose-dependent nephrotoxicity, a major side effect that restricts maximization of therapeutic effects. In clinical practice, approximately one-third of patients experience renal dysfunction after treatment with cisplatin (24). Recently, a number of platinum coordination complexes have been developed and studied to find related compounds that can demonstrate superior efficacy, fewer side effects, less cross-resistance, or improved pharmacological characteristics compared with the cisplatin parent compound. A few related compounds possessing similar or enhanced therapeutic efficacy have been found through preclinical screening; however, these compounds are also associated with nephrotoxicity of varying degrees of severity (10).

Cisplatin-induced nephrotoxicity is a complex process. After administration, cisplatin uptake is greatest within proximal tubular cells of the inner cortex and outer medulla. As a result, these segments are the major sites of cisplatin-induced renal injury, and the loss of tubular cells by necrosis and apoptosis is followed by infiltration of inflammatory cells and fibroproliferative changes. Cisplatin cytotoxicity is likely caused by a combination of multiple mechanisms, involving DNA damage (14), caspase activation (8), mitochondrial dysfunction (28), and formation of reactive oxygen species (13). However, the exact molecular and cellular mechanisms by which cisplatin induces nephrotoxicity remain unclear. Recently, results from multiple studies strongly implicate the importance of inflammatory mechanisms in the pathogenesis of cisplatin-induced nephrotoxicity; specifically through the recruitment of inflammatory cells, such as macrophages and leukocytes (7, 12, 34). It also appears that cisplatin induces increased renal expression of a variety of inflammatory chemokines and cytokines, such as tumor necrosis (TNF-α), transforming growth factor (TGF)-β, interleukin-1β (IL-1β), and intercellular adhesion molecular (ICAM)-1 (7). Reportedly, cisplatin-induced kidney injury depends on TNF-α, since TNF-α-deficient mice and TNF-α antibody-treated wild-type (WT) mice display resistance to cisplatin-induced kidney damage (23). Importantly, urinary levels of complement terminal complexes (C5b-9), the final end-product of complement activation, is increased in cisplatin-treated patients and is associated with nephrotoxicity (29). These findings, coupled with the important role of complements in immune and inflammatory responses, have led us to hypothesize that activation of the complement system may contribute to cisplatin-induced nephrotoxicity.

The complement system is one of the main effectors employed by the immune system in host defenses, and many by-products generated during complement activation are mediators of inflammation. The complement system consists of ~30 soluble- and membrane-bound proteins. In plasma, complement proteins interact with one another in one of three different sequential activation cascades known as the classical, alternative, and lectin pathways. Eventually, all three pathways converge, with complement proteins C3 and C5, into one terminal cascade that leads to formation of the membrane attack complex (MAC). The MAC is a macromolecular pore that has the ability to insert itself into cell membranes and lysed bacteria and heterologous cells (22). The small complement fragments generated during complement activation,
C3a and C5a, are known anaphylatoxins that induce several biological responses. Uncontrolled complement activation can lead to tissue inflammation or damage, which occurs in many immune-complex-mediated disease such as rheumatoid arthritis, asthma, liver diseases, and renal diseases (1, 4, 18, 22, 31). For example, activation of the complement system has been shown to contribute to the pathogenesis of ischemia-reperfusion renal injury, renal transplantation rejection, immune complex-mediated glomerulonephritis, lupus nephritis, IgA nephropathy, membrane-proliferative glomerulonephritis, and proteinuria-mediated renal damage (2, 4–6, 30, 32, 33, 36). In general, activation of the complement system induces MAC formation in tubular cells. The insertion of sublytic amounts of C5b-9 in the cellular membranes of tubular cells results in the production of proinflammatory cytokines that contribute to renal damage (2, 4–6, 30, 32, 33, 36). Conversely, the complement system may also exert protective effects in renal disease; in particular, early activation of the complement system results in clearance of immune complexes (4). At present, the role of the activated complement system in cisplatin-induced renal damage remains unknown.

To investigate whether complement activation contributes to cisplatin-induced nephrotoxicity, C5-deficient (C5KO), C5aR knockout mice (C5aRKO), and CD59ab knockout mice (CD59abKO) were used. Deficiency in C5 or C5a receptor (C5aR) proteins, but not CD59, which is a MAC regulator (21), attenuated cisplatin-induced nephrotoxicity. Moreover, administration of C5 or C5a restored cisplatin-induced nephrotoxicity in C5-deficient mice. These results indicate that C5a is an important pathogenic factor in cisplatin-induced renal injury, suggesting that C5aR blockade may be a novel strategy to prevent cisplatin-induced nephrotoxicity.

MATERIALS AND METHODS

Materials. Purified human C5a and C5 proteins were purchased from Complement Technology (Tyler, TX). The C5a and C5 proteins was >90% pure. These proteins were not treated to remove endotoxin contamination during the purification process. However, we checked endotoxin levels in the C5 and C5a protein preparations by using a kit from Sigma (St. Louis, MO). Appreciable endotoxin levels were not detected. Cisplatin was purchased from Sigma.

Treatment of mice with cisplatin, C5, or C5a proteins. Mice were treated with cisplatin (dissolved in saline, 20 μg/g body wt ip) or saline and killed 72 h postinjection. The kidneys were either immediately fixed with 4% formaldehyde and 2% glutaraldehyde or snap-frozen and kept at −80°C until examination. In some groups, C5KO mice were injected with human C5 (3 μg/g body wt ip) or C5a (1.7 μg/g body wt ip) proteins 10 min before cisplatin administration. The C5 and C5a proteins were dissolved in sterile PBS.

Measurement of serum complement activity with hemolytic assay. Rabbit erythrocytes were washed in PBS, and a 1% suspension was incubated with an equal volume of mouse anti-rabbit erythrocyte antiserum (1/100 dilution in PBS; 15 min at 37°C). The sensitized rabbit erythrocytes were washed with veronal buffered saline (VBS) and resuspended at 1% hematocrit. Fifty microliters of the cell suspension plus 50 μl of mouse serum (40% in VBS) were added to 96-well plates in triplicate, and the plates were incubated for 30 min at 37°C. Nonlysed cells were removed by centrifugation, and hemoglobin in the supernatant was measured as OD414. Percent hemolysis in each well was calculated as described previously (19).

Creatinine and blood urea nitrogen assay. Serum levels of creatinine (Cr) and blood urea nitrogen (BUN) were measured using kits obtained from Drew Scientific (Barrow-in-Furness, UK).

Western blotting. Anti-STAT3, anti-phospho-STAT3 (Tyr705), and anti-p NF-κB p105 (Ser933) antibodies were obtained from Cell Signaling Technology (Danvers, MA). Renal nuclear protein extracts were purified using an extraction kit (Thermo Scientific; Rockford, IL). The nuclear protein samples were mixed in Laemmli loading buffer, boiled for 5 min, and then subjected to SDS-PAGE (40 μg in each well). After electrophoresis, proteins were transferred onto nitrocellulose membranes and blotted against a primary antibody (1:1,000 dilution) for 16 h. Membranes were incubated with a secondary antibody linked with alkaline phosphatase (1:2,500 dilution) (Amersham Pharmacia Biotech, Piscataway, NJ) for 2 h, followed by incubation with enhanced chemiluminescent substrate (Amersham Pharmacia Biotech). Protein bands were visualized by scanning the membrane with PharosFx plus Molecular Imager (Bio-Rad Laboratories, Hercules, CA).

Immunohistochemistry. Paraffin-embedded sections were cut, deparaffinized, and hydrated by soaking in 100% xylene and descend-

Fig. 1. Complement activation during cisplatin-induced nephrotoxicity. A: serum complement activity from the mice treated with cisplatin was measured. Values are means ± SE. P < 0.001 vs. 0 time point. B: representative renal tissues stained with anti-C3bi/C3bi/C3c antibody. Enhanced staining was observed in the tissues from 24- and 48-h cisplatin-treated mice.
ing ethanol, followed by autoclave treatment. Next, sections were incubated in 0.3% H$_2$O$_2$ in PBS to block endogenous peroxidase activity. The sections were incubated with either anti-cleaved caspase 3 (1:100 dilution, Cell Signaling Technology), anti-MPO (Invitrogen, Carlsbad, CA), anti-nitrotyrosine (1:200 dilution, Cayman Chemical, Ann Arbor, MI), or anti-mouse C3b/iC3b/C3c antibody (Cell Sciences, Canton, MA) overnight at 4°C in a moist chamber. Biotinylated secondary antibodies and ABC Reagent were applied. Color development was induced by incubation with a DAB kit (Vector Laboratories, Burlingame, CA) for 3–5 min, and specific staining was visualized by light microscopy.

**Periodic acid-Schiff staining.** Following fixation of the kidneys with 10% formalin, renal tissues were sliced and stained with periodic acid-Schiff (PAS) for histological examination. Tubular damage in PAS-stained sections were examined by light microscopy (×200 magnification) and calculated as the percentage of cortical tubules showing epithelial necrosis (0 = normal; 1 = 10%; 2 = 10–25%; 3 = 26–75%; 4 = >75%). Five sections from each sample were

![Fig. 2](image-url)

**Fig. 2.** C5 deficiency protects mice from renal injury induced by cisplatin. Wild-type (WT) and C5 knockout (C5KO) mice were injected with cisplatin (20 mg/kg ip) or saline. Blood samples and kidney tissues were collected 72 h after cisplatin administration. A: serum levels of blood urea nitrogen (BUN) and creatinine were measured. B–F: representative renal tissues stained with periodic acid-Schiff (PAS; B), terminal deoxynucleotidyl transferase-mediated uridine triphosphate nick-end labeling (TUNEL; C), anti-cleaved caspase 3 antibody (D), MPO antibody (E), and anti-nitrotyrosine antibody (F) (magnification ×200). Tissue damage scores were quantified and are shown on the right. The number of TUNEL$^+$ cells, cleaved caspase 3$^+$ cells, and neutrophils was randomly counted in 10 fields (magnification ×40) per slide and are shown on the right. G: MDA levels were also measured. Values are means ± SE; n = 5 in saline group and n = 12 in cisplatin group (A); n = 5/group (B–G).
randomly selected for scoring by two independent investigators. Five to ten mice were used in each group.

Terminal deoxynucleotidyl transferase-mediated uridine triphosphate nick-end labeling. Terminal deoxynucleotidyl transferase-mediated uridine triphosphate nick-end labeling (TUNEL) staining was performed using an in situ apoptosis detection kit according to the manufacturer’s instructions (Chemicon International, Temecula, CA) and examined by light microscopy.

Malondialdehyde assay. Malondialdehyde (MDA) formation was used to quantify the tissue. Briefly, tissues were homogenized (100 mg/ml) in 1.15% KCl buffer, and homogenates (200 µl) were then added to a reaction mixture consisting of 1.5 ml of 0.8% thiobarbituric acid, 200 µl of 8.1% sodium dodecyl sulfate, 1.5 ml of 20% acetic acid (pH 3.5), and 600 µl of distilled H2O and heated for 45 min at 90°C. After cooling to room temperature, the samples were centrifuged at 10,000 g for 10 min, and the absorbance of the supernatant at A532 was measured with 1,1,3,3-tetramethoxypropane as an external standard. The level of lipid peroxides was expressed as nanomoles MDA per milligram protein.

Real-time PCR. Real-time PCR was performed as described previously (3, 9, 15). Briefly, total RNA was isolated from tissue homogenate using TRIzol reagents (Invitrogen) according to the manufacturer’s instructions. Total RNA (1 mg) was reverse-transcribed to cDNA using Super-Script II (Invitrogen). Target gene expression was quantified with iTaq Syber green Mix (Bio-Rad) using the Bio-Rad Chromo 4/Opticon system. Amplified samples from each set. RT-PCR analyses of the reference gene β-actin mRNA expression were performed in all samples. The results showed that β-actin mRNAs were comparable in all samples. Expression of all candidate gene mRNAs was normalized to β-actin mRNA level during calculation. Primer sequences for TNF-α, IL-1β, ICAM-1, caspase 3, caspase 8, and caspase 9 have been described previously (3, 9, 15).

Statistical analysis. Data are expressed as means ± SE (n = 5–10). To compare values obtained from two or more groups, Student’s t-test or one-way analysis of variance was performed. A value of P < 0.05 was considered significant.

RESULTS

Elevation of complement activity in serum and renal tissues after cisplatin treatment. Previous studies reported that the final end-product of complement activation is increased in cisplatin-treated patients and is associated with nephrotoxicity (29), suggesting complement is activated after cisplatin treatment in patients. Here, we measured the complement activity in serum and the cleaved C3 fragment deposition in the renal tissues of mice after cisplatin treatment. As shown in Fig. 1A, treatment of mice with cisplatin for 24 and 48 h significantly increased serum complement activity, which was returned to basal levels 72 h after cisplatin treatment. As shown in Fig. 1B, the cleaved C3 fragment deposition in the renal tissues of mice after cisplatin treatment was significantly increased compared to saline-treated groups. These results suggest that complement activity is increased after cisplatin treatment and is associated with nephrotoxicity.

Fig. 3. Administration of purified human C5 restores cisplatin-induced nephrotoxicity in C5KO mice. Purified human complement protein C5 (3 µg/g body wt) or PBS was injected intravenously 10 min before saline or cisplatin administration in C5KO mice. Blood samples and renal tissues were collected 72 h after saline or cisplatin administration. A: serum levels of BUN and creatinine were measured. B: tissue damage scores. C: renal tissues were stained with TUNEL, anti-cleaved caspase 3 antibody, or anti-MPO antibody. The number of positive cells was counted. D: MDA levels were measured. Values are means ± SE; n = 5/group.

Fig. 4. Administration of purified human C5a restores cisplatin-induced nephrotoxicity in C5KO mice. Purified human complement protein C5a (3.5 µg/g body wt) or PBS was injected intravenously 10 min before saline or cisplatin administration in C5KO mice. Blood samples and renal tissues were collected 72 h after saline or cisplatin administration. A: serum levels of BUN and creatinine were measured. B: tissue damage scores. C: kidney tissues were stained with TUNEL, anti-cleaved caspase 3 antibody, or anti-MPO antibody. The number of positive cells was counted. D: MDA levels were measured. Values are means ± SE; n = 5 in saline group and n = 6 in cisplatin group (A); n = 5/group (B and C).
posttreatment. Immunohistochemical analyses with the anti-C3b/iC3b/C3c antibody showed that levels of C3b/iC3b/C3c were markedly elevated in the renal tissues of mice treated with cisplatin for 24 and 48 h but not 72 h. Since the anti-C3b/iC3b/C3c antibody only recognizes the cleaved C3 fragments C3b, iC3b, and C3c but not native C3, the findings in Fig. 1B suggest that the complement is activated in renal tissues after cisplatin treatment. In addition, in vitro experiments showed that cisplatin itself did not directly activate complement (data not shown).

C5 deficiency protects mice from cisplatin-induced nephrotoxicity. To investigate the role of C5 in the pathogenesis of cisplatin-induced nephrotoxicity, C5KO mice were used. Basal levels of BUN and creatinine were similar between C5KO and their WT controls (Fig. 2A). No obvious renal histological changes were observed in C5KO mice compared with WT mice. Cisplatin treatment significantly elevated serum BUN and creatinine levels (Fig. 2A) and increased the percentage of damaged tubular cells (Fig. 2B) in WT mice. In contrast, C5KO mice were resistant to cisplatin-induced elevations in serum BUN and creatinine levels and renal tubular damage (Fig. 2). These results suggest that C5 plays a critical role in cisplatin-induced nephrotoxicity.

Apoptosis of renal tubular epithelial cells (8), formation of reactive oxygen species (13), and infiltration of inflammatory cells are well-known contributors to cisplatin-induced kidney dysfunction (11). We used TUNEL immunohistochemical staining and cleaved caspase 3 to assess renal tubular epithelial cell apoptosis and MPO, MDA, and nitrotyrosine to assess renal neutrophil infiltration and oxidative/nitrosative stress. In WT mice, cisplatin administration consistently and significantly increased the number

![Fig. 5. C5aRKO mice are resistant to cisplatin-induced renal dysfunction.](#)
of TUNEL-positive apoptotic cells (Fig. 2C), cleaved caspase
3-positive cells (Fig. 2D), and MPO-positive cells (Fig. 2E)
compared with C5KO mice. Higher levels of nitrotyrosine stain-
ing (Fig. 2F) and MDA (Fig. 2G) were also observed in WT mice.
In comparison, C5KO mice exhibited staining that was partially or
completed abrogated. These results further suggest that C5 is an
important mediator of increased apoptosis, inflammatory cell
infiltration, and oxidative/nitrosative stress observed with cispla-
tin-induced nephrotoxicity.

Administration of C5 or C5a restores cisplatin-induced
nephrotoxicity in C5KO mice. To confirm the role of C5 in
cisplatin-induced nephrotoxicity, C5KO mice were injected
with human C5 protein before cisplatin administration. As
shown in Fig. 3, A and B, serum BUN and creatinine levels, as
well as the number of damaged renal tubular cells, were
significantly higher after cisplatin administration in C5KO
mice pretreated with C5 compared with C5KO mice that did
not receive C5 pretreatment. Consistently, C5 pretreatment
increased the number of apoptotic cells and infiltrating neutro-
phils and increased MDA levels in the kidneys of cisplatin-
treated C5KO mice (Fig. 3C). Injection of C5 alone did not
cause elevation of BUN and creatinine (Fig. 3A) or obvious
histological changes in the kidney (data not shown).

During complement activation, C5 is cleaved into two
smaller fragments, C5a and C5b (1, 31). The C5b fragment
participates in the formation of MAC, while C5a functions as

Fig. 6. C5 or C5aR deficiency diminishes cisplatin-induced cytokine and caspase gene expression and STAT3/NF-
κB activation in murine kidneys. A–D: RT-PCR analyses were conducted to examine expression of cytokines and caspase genes in the kidneys 72 h after cisplatin or cisplatin plus C5 or C5a treatment. Expression levels
were normalized to β-actin expression levels and are expressed relative to saline-treated WT mice. Values are means ± SE (n = 4/group). A and B: *P < 0.05, **P < 0.01 compared with corresponding WT controls treated with cisplatin. C and D: *P < 0.05, **P < 0.01 compared with corresponding C5KO mice treated with cisplatin alone. E: nuclear extracts were isolated from the kidneys 72 h after cisplatin administration and used in Western blot analyses.
an anaphylatoxin. To determine whether C5a contributes to
cisplatin-induced renal injury, we injected C5KO mice with C5a
before cisplatin administration. Similar to administration
with C5, injection of C5a also restored cisplatin-induced nephor-
toxicity in C5KO mice. As shown in Fig. 4, BUN and creatinine
levels, tubular damage, the number of TUNEL-positive cells and
neutrophils, and MDA levels were significantly higher in C5KO
mice treated with C5a and cisplatin than mice treated with
cisplatin alone. Injection of C5a alone had little effect on elevation
of BUN and creatinine (Fig. 4A) and histological changes in the
kidney (data not shown).

Deficiency of C5a receptor protects against cisplatin-in-
duced renal dysfunction. The C5a fragment binds to corre-
sponding C5a receptors located on various immune cells. To
further identify the downstream pathway involved in C5a,
C5aRKO mice were used. At 72 h after cisplatin administra-
tion, C5aRKO mice had significantly reduced serum levels
of BUN and creatinine compared with WT mice (Fig. 5A).
There were also significantly fewer apoptotic cells, less infiltration
of neutrophils, lower nitrotyrosine levels, and lower MDA levels
in C5aRKO mice compared with WT mice (Fig. 5, B–G). This
suggests that the nephrotoxic effects of C5a are committed
after binding to C5a receptors, triggering downstream signaling
events.

C5a or C5aR deficiency diminishes cisplatin-induced renal
gene expression of cytokines and caspases, and activation of
p-STAT3 and NF-κB. It is well documented that cisplatin-
induced caspase activation and inflammatory chemokine and
cytokine release contribute to cisplatin-induced nephrotoxicity
(7, 8, 23). Here, we show that cisplatin administration upregulated
expression levels of TNF-α, IL-1β, ICAM-1, caspase 3, caspase
8, and caspase 9 in murine kidneys (Fig. 6A). In C5KO
and C5aRKO mice, this upregulation was blunted (Fig. 6, A
and B). Administration of purified human C5 or C5a restored
the upregulation of these cytokines and caspase genes in
cisplatin-treated C5KO mice (Fig. 6, C and D). Injection of C5
or C5a alone (without subsequent cisplatin treatment) had little
effect on these markers except for a slight increase in TNF-α
expression after C5a injection (Fig. 6, C and D).

Additionally, it has been established that activation of C5aR
by C5a results in IL-6/TNF induction and subsequent STAT3/
NF-κB activation (27). Hence, we examined whether STAT3/
NF-κB also becomes activated in the cisplatin-induced renal
injury model. As shown in Fig. 6E, cisplatin treatment elevated
renal expression of STAT3, p-STAT3, and NF-κB in WT
mice, but such induction was not observed in C5KO or
C5aRKO mice.

MAC formation does not contribute to cisplatin-induced
tenphrotoxicity. The principal regulator of complement MAC
assembly on cell membranes is CD59 (21). To exclude in-
volved of MAC formation in cisplatin-induced renal dys-
function, serum levels of BUN and creatinine were measured
72 h after cisplatin administration in WT and CD59abKO
mice. As shown in Fig. 7A, cisplatin treatment elevated serum
levels of BUN and creatinine in WT and CD59abKO mice
similarly. Immunofluorescence staining for MAC was also performed to assess the formation of MACs in kidneys after
cisplatin administration. As expected, WT mice and C5aRKO
mice had increased MAC deposition, while C5KO mice had
very low MAC deposition (Fig. 7B). No significant MAC
deposition was detected in WT mice not treated with cisplatin
(Fig. 7B).

DISCUSSION

For the first time, we demonstrate that C5KO and C5aRKO
mice are resistant to cisplatin-induced nephrotoxicity, and admin-
istration of purified C5 or C5a restores the sensitivity of C5KO
mice to cisplatin-induced nephrotoxicity. Using CD59ab double
knockout mice, we have excluded the role of MAC in this process.
Moreover, we also demonstrate that C5a or C5aR deficiency
diminishes cisplatin-induced cytokine and caspase gene expres-
sion, as well as p-STAT3 and NF-κB activation in kidneys from
mice. Taken together, these findings indicate that C5a contributes
to cisplatin-induced nephrotoxicity through a signaling pathway
downstream from C5a receptor binding.

Within the complement system, the C5 protein is a principle
mediator of host defenses (22). During activation of the com-
plement cascade, C5a and C5b are generated when C5 is
cleaved by its convertase. The C5a fragment is a potent
anaphylactic/chemotactic mediator that induces many proin-
flammatory activities, whereas C5b contributes to formation of
the C5b-9 MAC. Increased urinary levels of C5b-9 complexes
were found in cisplatin-treated patients and were associated
with nephrotoxicity (29). This suggests that injection of cis-
platin induces complement activation in patients. Here, we also
demonstrate that injection of cisplatin induced complement-
mediated renal damage through the C5 receptor pathway, not

![Fig. 7. Evidence for the lack of a role for the membrane attack complex (MAC) in cisplatin-induced nephrotoxicity. A: loss of restriction of MAC formation in CD59 KO mice does not enhance cisplatin-induced nephrotoxicity. WT and CD59ab double KO mice were injected with cisplatin (20 mg/kg ip). Blood samples were collected 72 h post-cisplatin administra-
tion. Serum levels of BUN and creatinine were measured. Values are means ± SE (n = 5/group). B: representative images of MAC immunofluorescence staining in mouse kid-
nneys 72 h after saline or cisplatin administration (magnification ×200). There was less MAC immunofluorescence stain-
ing in C5KO mice compared with WT and C5aRKO mice.](http://www.ajprenal.org/article-figures/502/7.png)
the formation of MAC. Furthermore, incubation of cisplatin with the serum in vitro did not lead to the complement activation (data not shown). This suggests that cisplatin itself does not directly activate complement and that activation of complement in vivo by injection of cisplatin may result from the secondary cisplatin-induced renal damage or the increased oxidative (MDA)-modified proteins (26). Normally, complement activation would cause complement consumption, leading to decreased serum complement activity. However, we found that the serum complement activity was increased after cisplatin treatment (Fig. 1). At present, the mechanisms by which administration of cisplatin causes complement activation but unexpectedly elevates serum complement activity remain unclear. It is plausible to speculate that injection of cisplatin causes renal damage and production of many proinflammatory cytokines (Fig. 6) (7), which could lead to the increased biosynthesis of the complement components in the liver and subsequently dominate the decreased serum complement levels during activation, leading to the elevated serum complement activity. Inhibition of C5a generation and C5b-9 formation by blocking C5 has been shown to ameliorate or prevent many inflammatory disorders associated with inappropriate complement activation, including renal ischemia-reperfusion injury (2, 6, 35) and glomerulonephritis (17). Here, we demonstrate that C5KO mice are resistant to cisplatin-induced renal injury, suggesting that activation of C5 is involved in the nephrotoxicity of cisplatin. Furthermore, we provide several lines of evidence suggesting that C5a specifically contributes to cisplatin nephrotoxicity. First, administration of purified human C5 or C5a restored cisplatin-induced renal injury in C5KO mice. Second, disruption of the C5aR gene also diminished cisplatin-induced renal injury. The C5a fragment exerts many proinflammatory and immunoregulatory actions after binding to C5aR, which belongs to the G protein-coupled receptor (GPCR) family, and is primarily expressed on the surface of immune cells such as macrophages, neutrophils, and T cells. Our findings show that C5aRKO and C5KO mice are resistant to cisplatin-mediated induction of proinflammatory cytokines. As administration of C5 or C5a restored inflammation responses in cisplatin-treated C5KO mice, the data collectively suggest that generation of C5a contributes to the induction of inflammation via binding to C5 receptor in cisplatin-induced renal injury, further supporting the conclusion that C5a participates in cisplatin-induced nephrotoxicity. In addition, administration of C5 or C5a alone did not cause nephrotoxic changes (Figs. 3 and 4), suggesting that other insults (e.g., C3bi-driven inflammation) in combination with C5 are required to induce nephrotoxicity.

The C5b fragment is generated when C5 is cleaved by C5 convertase. In turn, C5b contributes to the formation of MAC. It is well known that MAC formation contributes to many inflammatory kidney disorders, such as membranous nephropathy (16) and ischemia-reperfusion injury (2, 36). However, we provide two lines of evidence suggesting that MAC formation may not contribute to cisplatin-induced renal injury. First, CD59 is the major regulator of MAC formation, and cisplatin-induced renal injury was similar in both WT and CD59ab double knockout mice. Second, cisplatin-induced formation of MACs was observed in C5aRKO mice, but not in C5KO mice, while cisplatin-induced renal injury was diminished in both C5KO and C5aRKO mice, further indicating that MAC does not play a role in the renal toxicity of cisplatin.

In summary, our findings suggest that C5a contributes to cisplatin-induced nephrotoxicity through a signaling pathway downstream from C5a receptor binding, which is followed by induction of proinflammatory cytokines and neutrophil infiltration, resulting in renal damage. Additional studies to examine the role of C5a in human cisplatin induced-nephrotoxicity are warranted. The C5aR blockade could be a novel strategy in attenuating cisplatin-induced kidney injury.

GRANTS

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