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Osteopontin Expressed in Tubular Epithelial Cells Regulates NK Cell-Mediated Kidney Ischemia Reperfusion Injury

Zhu-Xu Zhang,*,†,‡,§ Kelvin Shek,¶ Shuang Wang,*,§ Xuyan Huang,*,§ Arthur Lau,*,§ Zqin Yin,*,§ Hongtao Sun,*,§ Weihua Liu,*,§ Bertha Garcia,*,†,‡ Susan Rittling,‖ and Anthony M. Jevnikar*,†,‡,§

Renal ischemia reperfusion injury (IRI) occurs after reduced renal blood flow and is a major cause of acute injury in both native and transplanted kidneys. Studies have shown diverse cell types in both the innate and the adaptive immune systems participate in kidney IRI as dendritic cells, macrophages, neutrophils, B cells, CD4+ NK+ cells, and CD4+ T cells all contribute to this form of injury. Recently, we have found that NK cells induce apoptosis in tubular epithelial cells (TECs) and also contribute to renal IRI. However, the mechanism of NK cell migration and activation during kidney IRI remains unknown. In this study, we have identified that kidney TECs express a high level of osteopontin (OPN) in vitro and in vivo. C57BL/6 OPN-deficient mice have reduced NK cell infiltration with less tissue damage compared with wild-type C57BL/6 mice after ischemia. OPN can directly activate NK cells to mediate TEC apoptotic death and can also regulate chemotaxis of NK cells to TECs. Taken together, our study’s results indicate that OPN expression by TECs is an important factor in initial inflammatory responses that involves NK cells activity in kidney IRI. Inhibiting OPN expression at an early stage of IRI may be protective and preserve kidney function after transplantation. The Journal of Immunology, 2010, 185: 967–973.

In the case of renal transplantation, injury that occurs during organ preservation and the early stages of surgery may lead to delayed graft function, which may then contribute to both early allograft loss and chronic allograft dysfunction (1). Renal ischemia reperfusion injury (IRI), which follows a period of reduced renal blood flow, is a major cause of acute renal failure from many etiologies. Indeed, severe injury can result in a very high mortality rate of 50% (2). Kidney IRI results from multiple molecular events. Although biological changes in tubular epithelial cells (TECs) play an important role in kidney injury and dysfunction, IRI is considered an Ag-independent inflammatory condition that involves multiple factors leading to tubular and endothelial dysfunction (3–5). We and others have demonstrated that TEC injury contributes to kidney dysfunction after exposure to inflammation and proinflammatory cytokines (6–8). After ischemia, oxidative stress induces cytokine and chemokine upregulation, which enhances the recruitment of inflammatory cells that are capable through the expression of cytokines, such as IFN-γ and TNF-α, of mediating both direct and indirect tissue injury (9, 10). Recent studies suggest in addition to classical involvement of innate immunity effectors, such as neutrophils, diverse cell types of the adaptive immune system can also participate in kidney IRI, including resident dendritic cells, B cells, CD4+ NK+ cells, CD3+ NK.1+ cells, and CD4+ T cells that also infiltrate into the kidney shortly after injury (11–21). Interestingly, as T and B cell-deficient Rag1−/− mice develop severe IRI, innate immune responses are clearly important in this type of kidney injury (22, 23). However, this does not exclude a significant role for diverse effectors in acute kidney injury.

Our recent study indicates that NK cells can directly kill TECs, and that NK cells contribute substantially to kidney IRI (24). NK cell killing may represent an important under-recognized mechanism of kidney injury that results from diverse forms of inflammatory injury, including transplantation. In that study, we demonstrated that activation of NK cells was essential to induce apoptosis of TECs in vitro. However, it is unknown what kidney factors activate NK cells or enhance their migration to the kidney after IRI.

Osteopontin (OPN), also known as the early T cell activation gene 1, is a secreted phosphorylated gp that is expressed in macrophages, T lymphocytes, and NK cells rapidly after activation (25–27). OPN acts as a proinflammatory cytokine and induces Th1-type cell immune responses (25, 28). It costimulates TCR-mediated T cell proliferation and prevents apoptosis (29). OPN augments production of IFN-γ and IL-12 but inhibits IL-10 production (28, 29). Furthermore, OPN has been shown to regulate Th1-mediated autoimmune demyelinating diseases in the rodent experimental autoimmune encephalomyelitis model (30, 31) as well as in infectious diseases (25, 28). As OPN levels increase after renal IRI, we considered whether OPN might play a role in NK cell activation or function in IRI.

Studies have reported that kidney cells produce a high level of OPN after injury as previously reviewed (32), suggesting OPN might play an important role in kidney injury after IRI. The impact of OPN in kidney injury could be both positive and negative, namely, inhibiting apoptosis or recruiting inflammatory cells. Indeed, a previous study reported that OPN deficiency significantly inhibited...
macrophage infiltration and increased apoptosis with less tubulointerstitial fibrosis (33). In contrast to this finding, others have found OPN deficiency elicited significantly higher serum creatinine levels and more prominent morphological injury than occurred with wild-type mice (34). As the role of OPN appears to have divergent functions, we were interested in determining the role of OPN in NK activation, chemotaxis, and kidney injury.

Materials and Methods

Animals

C57BL/6 (B6, H-2b) and Rag1−/− mice were purchased from The Jackson Laboratory (Bar Harbor, ME). OPN−/− were initially developed in 129 mice and backcrossed with C57BL/6 mice for nine generations (35). All animals were maintained in the animal facility at the University of Western Ontario using approved protocols and procedures.

TEC culture

The TEC line B6 NG1.1 was developed as previously described (6). Primary TECs were obtained from C57BL/6 or OPN−/− mice after digestion with collagenase (Sigma-Aldrich, St. Louis, MO) for 15 min and then sequentially sieved. All TECs were grown in complete K1 culture medium (DMEM:HamsF12, 50:50; Invitrogen-Life Technologies, Carlsbad, CA), supplemented with 5% bovine calf serum, hormone mix (5 μg/ml insulin, 1.25 μg/ml PGE1, 34 pg/ml triiodothyronine, 5 μg/ml transferrin, 1.73 ng/ml sodium selenite, and 18 ng/ml hydrocortisone), 100 U/ml penicillin (Invitrogen, Carlsbad, CA), 100 μg/ml streptomycin (Invitrogen), and 25 ng/ml epidermal growth factor. Cells were trypsinized before each passage. Proximal tubular phenotype of TECs was confirmed by staining with anti-CDS1, CD26, and E-cadherin.

Abs and reagents

Cells were characterized with fluorescent-conjugated mAb that specifically recognize CD3, CD4, CD8, NK1.1, DX5 (CD49b), and NKGD2 (eBioscience, San Diego, CA). Anti-OPN and recombinant mouse OPN (rmOPN) were purchased from R&D Systems (Minneapolis, MN).

Isolation of NK cells

NK cells were purified from C57BL/6 or Rag1−/− mouse spleens using anti-CD49b MACS beads selection after depleting CD3+ T cells (Miltenyi Biotec, Auburn, CA). Purity of NK cells was confirmed by flow cytometry (Invitrogen, Carlsbad, CA), 100 μg/ml streptomycin, and 25 ng/ml transferrin, 1.73 ng/ml sodium selenite, and 18 ng/ml hydrocortisone). 100 U/ml penicillin (Invitrogen, Carlsbad, CA), 100 μg/ml streptomycin (Invitrogen), and 25 ng/ml epidermal growth factor. Cells were trypsinized before each passage. Proximal tubular phenotype of TECs was confirmed by staining with anti-CDS1, CD26, and E-cadherin.

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Cytotoxicity assay

NK cells were purified by CD49b+ selection on a MACS beads column (Miltenyi Biotec), then activated in the presence of rmOPN (0.1–5 μg/ml) in RPMI 1640, supplemented with 10% FCS, penicillin (100 U/ml), streptomycin (100 μg/ml), glutamine (2 mM), sodium pyruvate (1 mM), HEPES (10 mM), and β-mercaptoethanol (0.5 mM). Activated or naive NK cells were used as effector cells in a killing assay. Cell death was confirmed with 7-aminoactinomycin-D (7-AAD) staining, according to the manufacturer’s protocol (BD Biosciences, San Diego, CA). Briefly, TECs were labeled with 0.5 μM CFSE (Molecular Probes/Invitrogen, Burlington, Ontario, Canada) for 10 min and washed three times with medium. The CFSE-labeled TECs were mixed with NK cells or cultured alone for 5 h. 7-AAD staining and flow cytometry were used to determine the percentage of the apoptotic/necrotic TECs by gating on CFSE+ cells.

Kidney reperfusion injury

Ischemia was induced in mice by clamping the left kidney for 45 min at 32 ℃ on a thermo-regulated pad. To assess function of the ischemic kidney, after the clamps were released, the right kidney was removed. Kidneys were sampled at different time points after being flushed with cold PBS. Sham controls were treated with the same operative procedure as in the injury group but kidneys were not clamped. Serum was collected for creatinine detection by a Jaffe reaction method with an automated CX5 clinical analyzer (Beckman, Fullerton, CA).

Quantitative real-time PCR

Total RNA was extracted from kidneys with a spin column, according to the manufacturer’s protocol (Qiagen, Mississauga, Ontario, Canada). cDNA pools were synthesized with the First Strand Synthesis System according to the manufacturer’s protocol (Stratagene, La Jolla, CA). Primers were designed using Primer Express, primer designing software from Applied Biosystems (Streetsville, Ontario, Canada). OPN, 5′-GCC TGT TTG GCA TTG CCT CTT C-3′ and 5′-CAC AGC ATG TGG CCG AAC G-3′; IFN-γ: 5′-CAT TGA AAG CCT AGA AAG TCT GC-3′ and 5′-TAG CGA TGC AAA TGC TTG AT-3′; IL-4: 5′-AGC TAG TTG TCA TTC TGC TTC TTC-3′ and 5′-AGG ATG TGT GCC ATG TAC TAC-3′; and β-actin: 5′-CCA GCC TTC CTT CCT GGG TA-3′ and 5′-CTA GAA GCA TTT GGG TTC CA-3′. The gene sequences were obtained from the www.ncbi.nlm.nih.gov database. Real-time quantitative PCR was performed on standardized quantities of cDNA using the Brilliant SYBR Green QPCR Master Mix kit, and amplified DNA products were generated and detected using the Mx4000 system (Stratagene). Each PCR amplification condition was set up in triplicate. β-actin amplification was used as the endogenous control. The normalized δ threshold cycle value and relative expression levels (2−δΔCt) were calculated according to the manufacturer’s protocol.

Histology and immunohistochemistry

Formalin-fixed kidney sections were stained with H&E, and injury was scored in a double-blinded fashion by a single pathologist, using an arbitrary scoring system (0: no change; 1+: <25% area change; 2+: 25–50% area change; 3+: 50–75% area change; 4+: >75% area change). Criteria for kidney injury included tubular necrosis, immune cell infiltration, tubular casts, and glomerular necrosis. Kidney sections were embedded in OCT compound and snap frozen in liquid nitrogen. Cryostat sections were fixed in acetone and stained with OPN Ab (R&D Systems) using a standard protocol. All slides were stained with a strepavidin-biotin immunoperoxidase and its substrate method by a standard method.
Migration assays
Assays with primary lymphocytes used transwells with 5-μm pores (Costar, Corning, NY). Briefly, NK cells (1 × 10^5) from C57BL/6 or Rag^−/− mice were labeled with 0.5 μM CFSE and placed in each upper chamber. Lower chambers contained complete medium with TECs or varying doses of rmOPN (R&D Systems). Transwell plates were incubated for 2–4 h. Total migrated cells were quantified by FACS to confirm CFSE positive NK cells. Quantification of migrated NK cells was determined as: migration index = migrated cells in the presence of TECs or OPN / migrated cells in the absence of TECs or OPN.

ELISA
OPN in TEC culture was detected using ELISA kits according to the manufacturer’s protocol (R&D Systems). B6 TECs or OPN^−/− TECs were seeded in 24-well plates the day before supernatants were collected. The supernatants were spun for 1500 rpm for 5 min before ELISA analysis. The obtained OD value was compared with serial diluted standard control of OPN (R&D Systems).

Statistical analysis
Data were compared using Student t test (Statview, Nesbit, MS) for unpaired values and one-way ANOVA for multigroup differences. The p values <0.05 were considered significant. Significance levels are provided in figures.

Results
Kidneys express high levels of OPN during IRI
Our recent study demonstrated that activated, but not naive, NK cells can induce apoptosis of TECs in vitro, and activated NK cells (CD69+) were present in kidneys during IRI (24). We were interested in identifying kidney factors that promote NK cell migration and as well as activate NK cells in the kidney after IRI. In general, chemokines and cytokines present within the kidney during early injury might alter NK cell function. IFN-γ, IL-2, and IL-15 are well-documented cytokines for activation and function of NK cells. However, as lymphocytes very quickly infiltrate into the kidney within 30 min after injury (14, 18) with a peak around 4 h after IRI (14, 24), we hypothesized that pre-existing or quickly released kidney factors in the kidney must participate in the initial NK cell migration after injury. We used real-time PCR to quantify various cytokines in the kidney after IRI. The expression of IL-15 and IFN-γ in the kidney was very low, and we were not able to detect significant levels of IL-2 and IL-7 after IRI (data not shown). In contrast, we have found that mRNA and protein expression of OPN was markedly increased within the kidney shortly after IRI (Fig. 1A). Importantly, in immunohistochemistry analysis, basal expression of OPN was evident in naive kidneys and, in keeping with previous findings (32), kidney expression of OPN increased after IRI (Fig. 1B).

FIGURE 2. OPN^−/− mice have less kidney IRI. A, Sera, collected after 1–5 d of IRI, were used for creatinine level analysis. Data represented average of 4 or 12 experiment mice in each group. B, Representative histology of IRI kidney. Data represented one mouse from each group. Similar data were obtained from all mice in the same group. The injury areas are depicted by arrows in the pictures. Magnification ×100. C, Kidneys were collected for pathology scoring analysis as described in Materials and Methods. Data represented average of 12 experiment mice in each group. Criteria for kidney injury, including tubular necrosis, immune cell infiltration, cast, and glomerular necrosis were scored (0: no change; 1+: <25% area change; 2+: 25–50% area change; 3+: 50–75% area change; 4+: >75% area change). Data were obtained using six mice from each group. *p < 0.05; **p < 0.01; ***p < 0.001.

FIGURE 3. OPN^−/− kidneys have less NK cells infiltrating after IRI. A, Kidneys were collected after 0, 4, and 24 h of IRI. After being digested by collagenase, the cells were suspended in 38% Percoll (Sigma-Aldrich) and overlaid on 72% Percoll, then centrifuged at 1000 × g for 30 min. Leukocytes were collected at interface of Percoll and counted before being stained with anti-CD45, anti-CD3, and anti-CD49b (DX5). Average number of CD3^+ CD49b^+ NK cells from each mouse before and after IRI. The results represent pooled data from 10–16 mice. B and C, Real-time PCR analysis of OPN expression in kidney. The kidneys were collected at different reperfusion time points after being perfused with PBS to remove blood from kidneys. β-actin amplification was used as the endogenous control. The normalized β threshold cycle value was calculated. The results represented average data from four mice at each time point. D and E, Real-time PCR analysis of IFN-γ and IL-4 expression in kidney. The kidneys were collected at different reperfusion time points after being perfused with PBS to remove blood from kidneys. β-actin amplification was used as the endogenous control. The normalized β threshold cycle value was calculated. The results represented average data from four mice at each time point.
**OPN deficiency leads to less kidney injury**

Previous studies have had diverging conclusions on the role of OPN in renal IRI as OPN deficiency either inhibited kidney injury (33) or elicited more prominent morphological damage (34). As many variables alter IRI susceptibility, including background strain and condition of ischemia, we tested IRI using OPN-deficient mice on the C57BL/6 background, in a standardized model of injury controlling clamp time and kidney temperature. All mice were treated with 45 min of ischemia, followed by reperfusion injury. As shown in Fig. 2A, OPN−/− mice consistently had less injury and better function, demonstrated by lower creatinine levels at 24 and 48 h compared with wild-type C57BL/6 mice (24 h: 75 ± 40 versus 104 ± 42 μmol/l, p < 0.01 and 48 h: 43 ± 28 versus 75 ± 31 μmol/l, p < 0.01). Consistent with serum creatinine levels, an independent, double-blind pathology analysis also indicated that OPN−/− kidneys had lower scores of injury compared with wild-type C57BL/6 mice (1.1 ± 0.6 versus 1.9 ± 0.8, p < 0.05, Fig. 2B, 2C). Collectively, our data indicate that OPN clearly promotes ischemic kidney injury.

**OPN−/− mice have less infiltrating NK cells after IRI**

Previous studies have demonstrated that CD4+ T cells and NK cells infiltrate into the kidney shortly after IRI, typically within 30 min to a few hours (11–18). However, infiltrating leukocytes appear to return to previous basal levels by 24 h after injury. We have also found that NK cells infiltrate into the kidney shortly after IRI and peak at around 4 h (24). We analyzed NK cell infiltration in OPN−/− and C57BL/6 male mice that underwent 45 min of ischemia, followed by reperfusion. Whole kidneys were digested with collagenase V and kidney cells were separated from infiltrating leukocytes using gradient Percoll, as described in *Materials and Methods*. Isolated total cells were analyzed by flow cytometry. Similar to previous reports and our study in wild-type mice, NK cells (CD3−CD49b+) increased quickly after 4 h; however, significantly fewer infiltrating NK cells were found in kidneys after 4 h in OPN−/− mice compared with C57BL/6 mice (Fig. 3A, 6.3 ± 1.6 × 10^4 versus 3.6 ± 1.5 × 10^4, p < 0.01).

OPN is considered to be a proinflammatory cytokine and induces Th1-type cell immune responses (25, 28). Less NK cell migration in OPN deficient kidney may be due to Th1 and Th2 balance alternation in the kidney. To test this possibility, we first analyzed expressions of Th1 (IFN-γ) and Th2 (IL-4) cytokines by real-time PCR. Expressions of IFN-γ and IL-4 were increased in kidneys of C57BL/6 and OPN−/− mice after IRI. IFN-γ is higher in wild-type B6 kidney than OPN−/− kidney at 4 h IRI (Fig. 3B), but there is no difference of IL-4 expression between the two groups (Fig. 3C). Serums were collected from IRI mice and used for ELISA. We did not find significant levels of IFN-γ and IL-4 proteins in

**FIGURE 4.** OPN regulates NK cell migration through an indirect effect. A. Production of OPN in TECs. The 1−4 × 10^5 C57BL/6 or OPN−/− TECs were placed in 12-well plates, and supernatants were collected after 24 h culture without any treatment. Production of OPN by TECs was confirmed by ELISA (R&D Systems). Data were pooled from three independent experiments. B. NK cells migration to TECs. NK cells (10^5) were purified from C57BL/6 mice by anti-CD49b MACS beads after depleting T cells, then labeled with 0.5 μM CFSE, and placed in each upper chamber of transwells with 5-μm pores (Costar). Lower chambers were seeded with TECs (2 × 10^5) developed from C57BL/6 or OPN−/− mice for 24 h. Neutralizing anti-OPN (0.1–10 μg/ml, R&D Systems) was added 30 min before NK cells were placed on top chambers. Total migrated cells were quantified by FACS to gate on CFSE positive NK cells after 2 h. Level of Migration Index NK/TEC was quantified as described previously. Data were averaged from four repeated experiments. C. NK cell migration toward OPN assay. Various concentrations of OPN were added into the lower chamber of the transwell. The 10^3 purified NK cells were added on the top wells. Two hours after, migration of NK cells was quantified, as described previously. Data were averaged from four different experiments. D. NK cell migration toward OPN-treated TEC assay. Various concentrations of rnOPN protein (R&D Systems) were added into the lower chamber of the transwell together with OPN−/− TEC (2 × 10^5) for 24 h. NK cells (10^5) were added on the top wells. Two hours after, migration of NK cells was quantified as described previously. Data were averaged from four repeated experiments. *p < 0.05; **p < 0.01; ***p < 0.001.
serum collected from B6 and OPN−/− mice after IRI (data not shown). We further examined Th1 and Th2 expressions in vitro. Primary cultured TECs from C57BL/6 and OPN−/− mice do not express IFN-γ and IL-4 by themselves. TECs from both C57BL/6 and OPN−/− kidneys could activate NK cells to produce low levels of IFN-γ and IL-4, but no significant differences were found between the two groups (Fig. 3D, 3E).

NK cells migrate to OPN-producing TECs

The finding that OPN−/− kidneys have less infiltrating NK cells, compared with wild-type mice, after injury suggests a role for OPN in promoting NK cell migration. We tested the effect of OPN on NK cell migration using an in vitro transwell system. We first determined whether TECs produce soluble OPN in vitro. TECs were purified and developed as described in Materials and Methods. As ELISA results showed in Fig. 4A, TECs produce high basal levels of OPN in supernatant without stimulation, whereas, as expected, OPN−/− TECs do not produce OPN protein. We then tested the capacity of OPN−/− TECs to attract NK cells in a transwell system. Naïve NK cells were purified and loaded on upper chambers without any further treatment. Lower wells were seeded with 2 × 105 TECs that were allowed to attach and culture for 24 h. Migration of CFSE-labeled NK cells was quantified by flow cytometry after 2 h incubation. NK cells were found to migrate toward wild-type C57BL/6 TEC, whereas much fewer NK cells were attracted by OPN−/− TEC (Fig. 4B). Interestingly, anti-OPN Ab (0.1, 1, and 10 μg/ml) consistently failed to inhibit NK cell migration rate to TECs (Fig. 4B, data not shown). These data would suggest that OPN expression by TECs was involved in NK migration but that the effect of OPN may be indirect. To further test this, we added recombinant OPN to lower wells without any TECs. NK cells did not show a significant increase of migration to rmOPN protein (Fig. 4C). Furthermore, when OPN protein was added to OPN−/− TECs at lower wells, migration of NK cells was increased compared with OPN−/− TECs alone (Fig. 4D).

OPN can activate NK cells that then mediate TEC death

Our data indicate OPN does play an important albeit a possible indirect role in NK cell migration to kidney. OPN can act as a proinflammatory cytokine and induces Th1-type cell immune responses (25, 28). It costimulates TCR-mediating T cell proliferation and prevents apoptosis (29). The capacity of OPN to activate NK cells is unknown. Therefore, we studied if OPN has a role in NK cell activation and subsequent TEC killing function. NK cells were purified from C57BL/6 mice and various doses of recombinant OPN (0.1–5 μg/ml) were added to the culture. NK cells were harvested 3 d after for analysis. Increased expression of granzyme B and CD69 on NK cells was found in OPN groups (Fig. 5A, data not shown) demonstrating that OPN can activate and potentially increase cytotoxic function of NK cells in vitro. In our previous study, we demonstrated that NK cells could induce TEC apoptosis in vitro after NK activation by IL-2 (24). We measured the cytotoxic function of OPN-activated NK cells in this model. NK cells were activated by OPN (5 μg/ml) for 4 to 5 d before being cocultured with TECs for 5 h. OPN-activated NK cells, but not nontreated NK cells, exhibited a strong TEC killing capacity in a dose-dependent manner (Fig. 5B).

Discussion

Both innate and adaptive immune systems participate in kidney IRI and the successful attenuation of IRI will need to consider DC, CD4+ T cells as well as macrophages and neutrophils. Our previous study has supported a role for NK cells in tubular cell injury in IRI, as they infiltrate into the kidney quickly after ischemia and can induce kidney TEC death and kidney injury in vitro and in vivo (24). In this study, we have identified OPN to play a key role in attracting and activating NK cells within the kidney during IRI. We have demonstrated that OPN expression by TECs and within kidneys regulates chemotactic activity of TECs for NK cell migration. Importantly and for the first time, we have demonstrated that OPN activates NK cells to enhance cytotoxic capacity to TECs in vitro. Consistent with its inflammatory functions, OPN-deficient mice had decreased injury and improved function after acute IRI. Hence, our data support a key role for OPN in promoting NK cell-mediated injury, with relevance to diverse forms of inflammatory kidney injury during transplantation that invariably results in ischemic injury.

In general, chemokines are early major mediators for inflammation, including promoting proinflammatory cytokine expression, upregulation of adhesion molecules, leukocyte infiltration, and activation. Various chemokines, including CC, CXC, and CX3C families, have been identified during kidney ischemia reperfusion injury (36). Hence, chemokines should have a significant effect on the migration of macrophages, neutrophils, NK cells, and T cells during kidney IRI. However, as demonstrated in our study and others, leukocytes infiltrate into the kidney as quickly as 30 min to 4 h (14, 14, 18, 24) suggesting that factors that promote injury must be rapidly upregulated or released within the kidney. Naïve kidneys express OPN as confirmed in the current study (Fig. 1) and monocytic/macrophage accumulation has been reported at OPN-expressing regions in the kidney (37–41), whereas OPN deficiency has a significant reduction in macrophage infiltration in the kidney after IRI (33). In other study models, OPN promotes accumulation of macrophages in obesity-induced adipose tissue (42) and OPN can trigger neutrophil infiltration into injured livers or into inflammation sites (43–45). Collectively, these studies suggest that OPN might be a candidate protein for modifying renal IRI by its effects on many
eral possible mechanisms that may help explain the difference in different mouse strains to IRI (53). In our model, OPN-null mice but may be due to the variable resistance and susceptibility of type mice, which would support a renoprotective function of phage infiltration and fibrosis in later stages (33). In contrast, previous report in which kidney function and morphology were experienced less acute kidney injury. This is in agreement with a pre-

test of renal injury. Indeed, in our study, OPN-deficient mice ex-
inhibiting OPN expression at an early stage of injury may limit the cytokines may contribute to injury by the activation of infiltrating and enhance tissue repair, OPN together with other chemokines and factors for tissue repair and can inhibit TEC apoptosis as well as participate in the regeneration and repair of TECs (32, 34, 47–52). The role of OPN in kidney injury is controversial. In the current study, we have demonstrated that OPN activates NK cells, which we show to be a prerequisite to induce TEC apoptotic death in vitro (Fig. 5). These data indicate that OPN can activate naive NK cells to induce apoptosis in TECs and suggest that OPN-expressing TECs may be capable of activating NK cells in situ to worsen kidney injury.

Although TECs may express OPN after IRI to prevent apoptosis and enhance tissue repair, OPN together with other chemokines and cytokines may contribute to injury by the activation of infiltrating leukocytes, including NK cells and T cells. In this scenario, inhibiting OPN expression at an early stage of injury may limit the extent of renal injury. Indeed, in our study, OPN-deficient mice experienced less acute kidney injury. This is in agreement with a previous report in which kidney function and morphology were preserved in OPN-null kidneys early in IRI with reduced macrophage infiltration and fibrosis in later stages (33). In contrast, OPN-null mice have reported to have more pronounced IRI (higher creatinine level and kidney histological abnormality) than wild-type mice, which would support a renoprotective function of OPN on TECs (34). The reason(s) for this discrepancy is unknown but may be due to the variable resistance and susceptibility of different mouse strains to IRI (53). In our model, OPN-null mice clearly had less severe kidney injury with reduced creatinine levels, less tissue damage, and fewer infiltrating NK cells. There are several possible mechanisms that may help explain the difference in results compared with previous reports (33, 34). Different genetic backgrounds may contribute to this difference in observed injury (53). This might result from pleiotropic functions of OPN as aforementioned. OPN is a proinflammatory cytokine that can enhance Th1-like immune responses (28–34). OPN also participates in tissue repair and prevents TEC apoptosis. Induction of inflammatory responses and contribution of OPN to antiapoptosis/tissue repair may vary in mice with different genetic backgrounds. Hybrid strains in particular with mixed MIC genetic backgrounds (129xC57BL/6 F2 versus 129xBlack Swiss) display various degrees of IRI. In this study, we used OPN-null mice on a near-complete C57BL/6 background to control for this variability between control and experimental groups to more clearly define the role of OPN in kidney injury. In addition, the amount of experimental injury is altered by organ temperature. In comparison with a previous study (33), we were able to obtain a reduced level of injury by applying a lower temperature, and this may have made differences in injury levels more apparent.

In summary, the current study demonstrates that OPN augments NK cell migration, activates NK cells, and enhances NK cell-mediated TEC apoptotic death. These effects would be expected to potentiate inflammation and injury in the kidney during ischemia and reperfusion. As OPN-deficient mice have less infiltrating NK cells and less tissue damage after IRI, inhibition of OPN expression at an early stage of injury may be beneficial in preserving kidney function during inflammation.

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Disclosures

The authors have no financial conflicts of interest.

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