Depletion of kidney CD11c⁺ F4/80⁺ cells impairs the recovery process in ischaemia/reperfusion-induced acute kidney injury

Myung-Gyu Kim*, Chang Su Boo*, Yoon Sook Ko, Hee Young Lee, Won Yong Cho, Hyoung Kyu Kim and Sang-Kyung Jo

Division of Nephrology, Department of Internal Medicine, Korea University Anam Hospital, Korea University College of Medicine, Seoul, Korea

Correspondence and offprint requests to: Sang-Kyung Jo; E-mail: sang-kyung@korea.ac.kr

*These authors contributed equally to these experiments.

Abstract

Background. Recent studies provided evidence of the potential role of CD11c⁺ F4/80⁺ dendritic subset in mediating injury and repair. The purpose of this study was to examine the role of kidney CD11c⁺ F4/80⁺ dendritic subset in the recovery phase of ischaemia/reperfusion injury (IRI).

Methods. Following ischaemia/reperfusion (I/R), liposome clodronate or phosphate buffered saline (PBS) was administered, and on day 7 biochemical and histologic kidney damage was assessed. Activation and depletion of CD11c⁺ F4/80⁺ dendritic subset were confirmed by flow cytometry. Isolation of kidney CD11c⁺ cells on days 1 and 7 with in vitro culture for measuring cytokines was performed to define functional characteristics of these cells, and adoptive transfer of CD11c⁺ cells was also done.
**Results.** Following kidney IRI, the percentage of CD11c<sup>+</sup> F4/80<sup>+</sup> kidney dendritic cell subset that co-expresses maturation marker increased. Liposome clodronate injection after I/R resulted in preferential depletion of CD11c<sup>+</sup> F4/80<sup>+</sup> kidney dendritic subset, and depletion of these cells was associated with persistent kidney injury, more apoptosis, inflammation and impaired tubular cell proliferation. CD11c<sup>+</sup> F4/80<sup>+</sup> cell depletion was also associated with higher tissue levels of pro-inflammatory cytokines and lower level of IL-10, indicating the persistence of inflammatory milieu. Isolated kidney CD11c<sup>+</sup> cells on day 7 showed different phenotype with increased production of IL-10 compared with those on day 1. Adoptive transfer of CD11c<sup>+</sup> cells partially reversed impaired tissue recovery.

**Conclusion.** Our results suggest that kidney CD11c<sup>+</sup> F4/80<sup>+</sup> dendritic subset might contribute to the recovery process by dynamic phenotypic change from pro-inflammatory to anti-inflammatory with modulation of immune response.

**Keywords:** acute kidney injury; dendritic subset; liposome clodronate; recovery

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**Introduction**

Acute kidney injury (AKI) is a significant health problem with a high morbidity and mortality, and ischaemia/reperfusion injury (IRI) is the leading cause of AKI in native or allograft kidneys [1]. Inflammation is well-known to play a pivotal role in the pathophysiology of kidney IRI [2–4], and recently immune responses have been identified as important for mediation of IRI [5–8].

Dendritic cells (DCs) are the major antigen-presenting cells of the immune system with the unique capacity to trigger naive T cell responses, linking innate and adaptive immune responses [9,10]. Although the precise role of kidney DCs in IRI has not been completely addressed, recent studies have demonstrated the activation of resident kidney DCs in the early phase of IRI [6,11] and also the participation of DCs in kidney injury by secreting tumour necrosis factor-alpha (TNF-α) and other pro-inflammatory cytokines [12]. However, the ability of DCs to induce immune tolerance or tissue protection in various types of experimental injury models has also been reported recently. DCs freshly isolated from the liver after IRI exhibit a mature phenotype with an inhibitory profile of increased IL-10 and reduced IL-12 production [13]. In addition to the traditional function as inducers of adaptive immunity, kidney DCs stimulate the production of IL-10 in infiltrating CD4<sup>+</sup> Th1 cells and attenuate nephrotoxic nephritis [14]. In an asthma model, pulmonary DCs capture airborne allergens and ameliorate disease by inducing CD4<sup>+</sup> regulatory 1-like cells, with IL-10 production [15]. Although these findings strongly suggest that CD11c<sup>+</sup> tissue-resident DCs might have a heterogenous phenotype with different roles in injury and repair, complete differentiation of DCs from macrophage in vivo is very difficult because of overlapping of surface markers and/or even function. In the current study, we investigated the role of CD11c<sup>+</sup> F4/80<sup>+</sup> kidney dendritic subset in repair and regeneration processes following ischaemia/reperfusion (I/R).

We showed that the kidney dendritic subset was activated following I/R, and depletion of these cells in the recovery phase was associated with persistent kidney damage. The pro-inflammatory cytokines, interferon-γ (IFN-γ), interleukin-6 (IL-6) and TNF-α expression increased significantly, and IL-10 level was significantly lower in CD11c<sup>+</sup> F4/80<sup>+</sup> dendritic subset-depleted kidney tissue with persistent tissue inflammation. A significantly higher level of IL-10 was also observed in the supernatant of the CD11c<sup>+</sup>-enriched cell population from kidneys harvested on day 7 following I/R compared to those from kidneys harvested on day 1, suggesting that kidney dendritic subset in the recovery phase of IRI had an anti-inflammatory phenotype and possibly contributed to the recovery process. Adoptively transferred spleen-derived CD11c<sup>+</sup> cells were identified in the kidney, and partially-reversed persistent tissue injury was observed in liposome clodronate-treated mice.

These data suggest that CD11c<sup>+</sup> F4/80<sup>+</sup> kidney dendritic subset plays an important role in recovery or regeneration processes from IRI by modulating immune responses toward anti-inflammation. Further studies identifying new mechanisms mediating recovery or regeneration will help open a new avenue to the treatment of AKI.

**Materials and methods**

**Animals**

Six-to-eight week-old male C57BL/6 mice (20–25 g) and Balb/c mice (20–25 g) were purchased from Orient (Charles River Korea, Seoul, Korea) and had free access to water and chow before manipulation. Experiments were conducted in accordance with the criteria established by the Animal Care Committee of Korea University for the care and use of laboratory animals in research. Animals were anaesthetized with the intraperitoneal injection of 15 mg/kg of ketamine and 2.5 mg/kg of xylazine and subjected to bilateral renal pedicle clamping for 28 min. The animals were kept at a constant temperature (37°C) during the procedure and allowed to recover. After the clamps were removed, reperfusion of the kidneys was visually confirmed. A sham operation was performed in a similar manner, except for clamping of the renal pedicles. On day 7 after I/R, animals were sacrificed, blood was collected by intracardiac puncture and both kidneys were processed for molecular and histologic examinations.

**Liposome clodronate preparation and in vivo depletion of kidney dendritic subset**

Clodronate was purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA), and liposome-encapsulated clodronate was prepared according to methods described previously by Van Rooijen et al. [16]. Animals were injected intravenously with 10 μg of liposome clodronate or PBS on days 1 and 3 after reperfusion.

**Biochemical analysis**

Three hundred microlitres of blood was collected on day 7 after reperfusion, and serum creatinine was measured using a Hitachi 747 automatic analyzer.

**Kidney tissue processing and flow cytometric analysis of kidney leukocytes**

Kidney suspensions were prepared from mice subjected to IRI, IRI + DC-depletion or sham operation on day 7. Kidneys were minced and incubated with collagenase type IV (10 μg/ml, Sigma-Aldrich) and DNase (Sigma-Aldrich) in PBS buffer with ethylenediaminetetraacetic acid (EDTA) (2 mM) for 20 min at 37°C. The digested kidney suspension was filtered through a 100-μm BD Falcon cell strainer (BD Bioscience, San Diego, USA).
CA, USA) and then passed through a cotton column treated with 10% FBS and then centrifuged at 3000 r.p.m./min for 5 min. The cell pellet was washed with PBS containing 1% BSA and 0.1% sodium azide and resuspended. Antibodies were purchased from eBioscience (San Diego, CA, USA; CD45, CD11c, F4/80, CD80, CD86 and Ly6G) and BD Bioscience (MHC II and AAD). Fresh kidney suspension was first incubated with anti-mouse CD45-FITC for 30 min on ice, and after washing, the cells were stained for 15 min on ice with fluorochrome-labelled monoclonal antibodies against CD11c, MHC II, CD80, CD86, Ly-6G and AAD. Four-colour fluorescence flow cytometric analyses were performed (FACSCalibur™, BD Bioscience) to determine the phenotypes of renal DC subset and other leukocytes, and the data were analysed with the FlowJo program (Tree Sta Inc., Ashland, CA, USA).

**Histologic examination**

Paraformaldehyde (4%)-fixed and paraffin-embedded kidney tissues were stained with periodic acid-Schiff (PAS). Histologic changes in the corticomedullary junction and outer medulla were evaluated semi-quantitatively. The number of neutrophils and apoptosis was counted and graded in a scale from 0 to 2. Degree of tubular cell necrosis and loss of PAS-positive tubular brush borders as well as cast formation were also graded from 0 to 2 according to the percentages per HPF. The mean score of these five criteria was compared between the groups. Immunohistochemical detection of F4/80+ or CD11c+ cells was performed on paraformaldehyde (4%)-fixed and paraffin-embedded kidney sections using monoclonal antibody against F4/80 (Serotec, Oxford, UK) or CD11c (Abcam, MA, USA). Briefly, after deparaffinization, endogenous peroxidase activity was blocked with 0.5% hydrogen peroxidase. Sections were blocked for 10 min with goat serum, followed by incubation with primary antibody for 1 h at room temperature (1:100). Specific labelling was detected with a biotin-conjugated rabbit anti-rat immunoglobulin G and avidin–biotin–peroxidase complex (Vector Laboratories, Burlingame, CA, USA). Proliferating cell nuclear antigen (PCNA) staining was also performed to evaluate the cell proliferation, and PCNA-positive cells in the corticomedullary junction and outer medulla were measured by counting 8–10 high power field (HPF) (×200) per section and the mean number was compared.

**Detection of apoptosis**

Apoptotic cells in paraffin-embedded kidney tissue sections were identified using ApopTagPlus (Intergen, Purchase, NY, USA) according to the manufacturer’s protocol. The number of apoptotic cells in the corticomedullary junction and outer medulla was semi-quantitatively measured by counting 8–10 HPF (×200) sections, and the mean number of terminal deoxynucleotidyl transferase nick end labelling (TUNEL)-positive cells was compared.

**Quantitation of cytokines/chemokines by cytometric bead array**

Quantitation of various cytokines and chemokines in kidney tissue was done using cytometric bead array (CBA). A mouse inflammation kit (BD

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**Fig. 1.** Ischaemia/reperfusion-induced maturation of kidney CD11c+ cells. Cell suspensions from sham operated, 24 h, 72 and 7 days after I/R were stained for CD45 and then subsequently for CD11c in combination with a panel of antibodies against DC maturation markers (MHC II, CD80 and CD86). (A) Flow cytometric analysis of MHC II expressing CD11c-DCs at different time points after I/R. (B, C, D). The percentage of CD11c-DCs that co-expressed surface activation markers MHC class II, CD80 and CD86 was expressed as mean ± SE, respectively. *P < 0.05 compared with sham kidney. **P < 0.05 compared with I/R day 1. n = 4–5 per group.
CD11c⁺ F4/80⁺ kidney dendritic subset contributes to recovery in AKI

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Bioscience) was used according to the manufacturer’s instructions to simultaneously detect mouse IL-12p70, TNF-α, IFN-γ, MCP-1, IL-10 and IL-6 from total kidney extracts [19]. Briefly, a mixture of six capture bead populations (50 μl) with distinct fluorescence intensities (detected in FL3) coated with antibodies specific for the above cytokines/chemokines was mixed with each sample from the kidney homogenates/standard (50 μl). Additionally, PE-conjugated detection antibodies (detected in FL-2; 50 μl) were added to form sandwich complexes. After 2 h of incubation, the samples were washed once (200 g, 5 min) and resuspended in 300 μl of wash buffer before acquisition in a FACS® flow cytometry (FACS® Calibur®). Following acquisition of data, the sample results were analysed using CBA software (BD Biosciences). Standard curves were generated for each cytokine using the mixed cytokine/chemokine standard provided by the kit. The concentration for each cytokine in cell supernatants was determined by interpolation from the corresponding standard curve and normalized according to protein concentrations. The range of detection was 20–5000 pg/ml for each cytokine. Mean cytokine expression level normalized to gram protein in each group was expressed as mean ± SE and compared between groups.

**Isolation of CD11c⁺ kidney dendritic subset and measurement of cytokines and chemokines**

CD11c⁺ kidney dendritic subset was isolated from mouse kidneys obtained on days 1 or 7 after reperfusion or from a sham operation. Briefly, the kidney single cell suspension was digested with type IV collagenase (Sigma-Aldrich) and DNase (Sigma-Aldrich) at 37°C for 20 min and then subjected to 10 min of centrifugation at 40 g. The cells in supernatant were resuspended in Hank’s balanced salt solution, layered on Ficoll and centrifuged at 1000 g for 20 min. Mononuclear cell fraction from the low density interface cell fraction was collected, passed through a cotton column treated with 10% fetal bovine serum (FBS) and washed with PBS. The final cell suspension was incubated with anti-mouse CD11c-coated magnetic beads (Miltenyi Biotec, Bologna, Italy) and then positively selected, isolating the CD11c⁺-enriched cell population. The purity of the CD11c⁺ cells was identified using flow cytometry (~90%). Isolated kidney CD11c⁺-enriched cell populations at different reperfusion times were incubated in 96-well plates overnight, and cytokine and chemokine concentrations in the supernatants were analysed using CBA.

**Adoptive transfer of CD11c⁺ cells after depletion of kidney CD11c⁺ F4/80⁺ cells**

CD11c⁺ cells were isolated from normal mouse spleen. Briefly, spleen suspension was filtered through a 100 μm BD Falcon cell strainer and purified by density centrifugation using Ficoll at 90 g for 20 min. Cell suspension from the low density interface was incubated with a cocktail of biotin-conjugated monoclonal antibodies against CD90, CD45R, CD49b, CD8a, CD3 and Ly-6G (Miltenyi Biotec) and negatively selected. The isolated splenic cells were positively selected, isolating the CD11c⁺ cell population. The purity of CD11c⁺ cells was identified using flow cytometry (~90%). CD11c⁺ cells (5 × 10⁵) were adoptively transferred into mice on day 5 after liposome clodronate injection following IRI, and kidney tubular injury score and tissue cytokine levels by CBA were compared between groups.

**Labelling of spleen- derived CD11c⁺ cells with Dil and in vivo tracking**

Isolated CD11c⁺ cells were labelled with CellTracker™ CM-Dil (Molecular Probe, Invitrogen, USA) with the final concentration of 25 mg/ml Dil at 37°C for 15 min and adoptively transferred into mice on day 5 after IRI after liposome clodronate injection. Mice were perfusion fixed with 4% paraformaldehyde, and kidney, lung, spleen and liver were proceeded with sequential fixation with 5% sucrose in PBS overnight at 4°C, 30% sucrose in PBS for 8 h at 4°C and frozen at −70°C. Fluorescence microscopy was applied on 4-8 m cryosections using rhodamine filter.

**Statistical analysis**

All data were expressed as the mean ± SE and analysed using SPSS, version 13.0. A non-parametric Mann–Whitney test was employed to determine the statistical significance between the groups; a P-value of <0.05 was considered to be statistically significant.
Results

**CD11c^+ F4/80^+ kidney dendritic subset in late reperfusion phase displays a mature phenotype**

We examined the phenotypic change of the kidney CD11c^+ dendritic subset after IRI. To examine the maturation status of renal dendritic subset, flow cytometric analysis was performed. Single cell suspensions prepared from the kidneys 24, 72 h and 7 days after I/R and sham operations were stained for the pan-leukocyte marker, CD45, in combination with the DC marker, CD11c, and the DC maturation markers, MHC II, CD80 and CD86. Following reperfusion, the total number of CD11c^+ cells increased at 24 h, with only 27% of the cells co-expressing MHC II. However, at 72 h after reperfusion, 51% of the CD11c^+ cells co-expressed MHC II, indicating maturation of CD11c^+ kidney dendritic subset (Figure 1A, B). Increased surface expression of MHC II in CD11c^+ cells persisted until day 7 after I/R. A similar pattern of expression was observed in CD11c^+ kidney dendritic subset that expressed CD80 or CD86, suggesting that I/R induced maturation of DC subset at late reperfusion phase (Figure 1C and D).

**Liposome clodronate administration depletes CD11c^+ F4/80^+ kidney dendritic subset**

Intravenously injected liposome clodronate is well-known to deplete monocytes-macrophages systemically, and previous studies have demonstrated that monocyte-macrophage depletion before I/R significantly reduces kidney injury [16,17]. We first examined the effect of delayed administration of liposome clodronate on F4/80^+ or CD11c^+ cells. Immunohistochemical staining of kidneys showed that liposome clodronate treatment after I/R resulted in a marked decrease of F4/80^+ cells and also CD11c^+ cells (Figure 2). Because F4/80 or CD11c is known to be expressed in both macrophages and DCs [18], we tried to define the phenotype of cells that were depleted by liposome clodronate injection more specifically by performing the flow cytometric analysis of kidney single cell suspension.

We observed that liposome clodronate injection 24 and 72 h after I/R resulted in more preferential depletion of CD11c^+ F4/80^+ kidney dendritic subset rather than CD11c^- F4/80^+ macrophages (Figure 3).

**Depletion of DCs impairs the recovery process from IRI**

We next examined the effect of CD11c^+ F4/80^+ kidney dendritic subset depletion on the recovery or regeneration process from IRI. We first evaluated the biochemical and histologic renal damage on day 7 following I/R. The serum creatinine or blood urea nitrogen (BUN) level was not significantly different between the liposome clodronate- and PBS-treated groups (Figure 4F). However, histologic examination showed persistent tubular injury characterized by loss of brush border, tubular cell necrosis, dilatation of tubules and cast formation in the corticomedullary junction and outer medulla in liposome clodronate-treated animals compared to PBS-treated animals (Figure 4A and B). Semi-quantitative assessment of histologic damage demonstrating persistent kidney injury and inflammation from IRI in liposome clodronate-treated mice is shown in Figure 4E. We counted the number of neutrophils and ap-
optosis and graded in a scale from 0 to 2. Degree of tubular cell necrosis and loss of PAS-positive tubular brush borders as well as cast formation were also graded from 0 to 2 according to the percentages per HPF. The mean score of these five criteria was compared between the groups. In order to confirm that these findings are not confined to one mouse strain, we repeated these experiments in a different strain (Balb/c) and also observed impairment of kidney recovery in kidney dendritic subset-depleted animals (Figure 4C, D and E).

**Effect of CD11c\(^+\) F4/80\(^+\) kidney dendritic subset depletion on apoptosis**

Although apoptosis at early reperfusion phase is a well-known feature of kidney dysfunction in IRI, the precise time course of apoptosis in the recovery phase has not been clearly demonstrated. We found that there was still a significant increase in apoptosis on day 7 following IRI. However, the number of apoptotic cells in CD11c\(^+\) F4/80\(^+\) kidney dendritic subset-depleted kidneys was significantly higher, suggesting that depletion of dendritic subset was associated with the persistent presence of apoptosis, contributing to kidney injury (Figure 5A–C).

**Effect of CD11c\(^+\) F4/80\(^+\) kidney dendritic subset depletion on cell proliferation after IRI**

To examine the effect of dendritic subset depletion on cell proliferation after IRI, we performed immunohistochemical study of PCNA expression (Figure 6A and B). The number of PCNA-positive cells in kidneys from liposome clodronate-treated mice was significantly lower than PBS-treated mice, showing impairment of tubular cell regeneration (Figure 6C).

**Depletion of CD11c\(^+\) F4/80\(^+\) kidney dendritic subset was associated with persistent inflammation with increased tissue levels of TNF-\(\alpha\), IL-6 and INF-\(\gamma\) and with decreased levels of IL-10**

Using CBA, we examined the effect of kidney dendritic subset depletion on various tissue cytokine and chemokine
expression in the recovery phase after IRI. A mouse inflammation CBA kit (BD Bioscience) was used. Compared to PBS-treated animals, INF-γ, TNF-α and IL-6 were significantly higher, whereas the anti-inflammatory cytokine, IL-10 level was significantly lower in liposome clodronate-treated animals, suggesting that dendritic subset depletion was associated with a persistent inflammatory milieu (Figure 7A). We also examined the number of neutrophils by flow cytometric detection of CD45+/Ly6G+ cells. Neutrophils are known to participate in kidney injury by releasing various mediators, and we found that kidney dendritic subset depletion was associated with an increased number of kidney neutrophils in the recovery phase (Figure 7B).

CD11c+ kidney dendritic subset changed their phenotype from pro-inflammatory to anti-inflammatory

To gain better insight of the direct causal relationship between depletion of kidney dendritic subset and persistent inflammation and injury following I/R, we examined the phenotype of kidney dendritic subset at various time points following I/R by isolating CD11c+ cells from a kidney single cell suspension and by measuring cytokine and chemokine secretion. TNF-α and IL-6 levels in the supernatants of a CD11c-enriched cell culture from 24-h reperfused kidneys increased significantly compared to the one from sham-operated kidneys, indicating that the phenotype of kidney dendritic subset in the early reperfusion period was pro-inflammatory. However, CD11c+ cells isolated from day-7 reperfused kidneys secreted significantly higher amounts of IL-10 compared to day-1 reperfused kidneys, suggesting the conversion of the phenotype into anti-inflammatory phenotype (Figure 8). TNF-α and IL-6, however, were not different between day 1 and day 7.

Adoptive transfer of CD11c+ splenic DCs results in partial reversal of tissue injury with decreased levels of pro-inflammatory cytokines

CD11c+ splenic DCs were isolated and adoptively transferred into mice after kidney dendritic subset depletion. The purity of CD11c+ splenic DCs was more than 90% by flow cytometric analysis (Figure 9D). In histologic examination, persistent tubular injury was partially recovered in CD11c+ transferred mice, suggesting a beneficial effect of CD11c+ cells on kidney recovery following I/R (Figure 9A–C). The persistently increased tissue level of TNF-α and IL-6 that was observed in dendritic subset-depleted animals was also partially reversed by CD11c+ cell reconstitution (Figure 9E and F).

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**Fig. 4.** Effects of depletion of CD11c+ F4/80+ cells on renal histology and function in recovery phase after IRI. Mice were intravenously injected with either PBS or liposome clodronate on days 1 and 3 after I/R. Representative pictures of PAS-stained section of kidney from mouse injected with PBS (A, C) or liposome clodronate (B, D) (PAS, ×200). (E) Semi-quantitative scoring of histologic injury. The number of neutrophils and apoptosis was counted and graded in a scale from 0 to 2. Degree of tubular cell necrosis and loss of PAS-positive tubular brush borders as well as cast formation were also graded from 0 to 2 according to the percentages per HPF. The mean score of these five criteria was compared between the groups. (F) BUN (mg/dl) on day 7 following I/R. *P < 0.05 compared with sham, **P < 0.05 compared to I/R + PBS. n = 5 per group.
Transferred CD11c⁺ cells were identified in I/R kidneys

Transferred CD11c⁺ cells were occasionally identified in juxtaposition to tubular epithelial cells in I/R day 7 kidneys. In addition to kidney, those cells were also seen in liver, spleen and lungs. However, we could not detect Dil-positive cells in normal kidneys or any other organs of mice who were injected with non-labelled cells (Figure 10).

Discussion

In the present study, we demonstrated the following: (i) IRI induced kidney dendritic subset maturation by demonstrating an increased percentage of CD11c⁺ cells that co-express MHC II, CD80 and CD86; (ii) intravenous administration of liposome clodronate after I/R resulted in more preferential depletion of CD11c⁺ F4/80⁺ kidney dendritic subset on day 7, and depletion of these cells was associated with persistence of tissue injury, inflammation and decreased cell proliferation; (iii) impairment of the recovery process was accompanied by higher tissue levels of pro-inflammatory cytokines and lower levels of IL-10; (iv) in vitro studies using kidney-derived CD11c⁺ cell cultures showed that kidney CD11c⁺ cells in the recovery

Fig. 4. (continued).

Transferred CD11c⁺ cells were identified in I/R kidneys

Fig. 5. Effect of depletion of CD11c⁺ F4/80⁺ cells on apoptosis in recovery phase after IRI. Mice were intravenously injected with either PBS or liposome clodronate on days 1 and 3 after IRI. Apoptosis was detected by TUNEL methods on day 7 after IRI. (A) I/R + PBS and (B) I/R + liposome clodronate-treated kidneys, TUNEL staining, × 200. (C) The mean number of TUNEL-positive cells was expressed as mean ± SE. *P < 0.05 compared with I/R + PBS. n = 4–5 per group.
phase of IRI secrete higher amounts of IL-10 compared to those in the early reperfusion period, suggesting the change to an anti-inflammatory phenotype and also the important role of kidney CD11c+ dendritic subset in the recovery process by modulating the inflammatory response; (v) in vivo study, reconstitution with CD11c- splenic DCs resulted in partial reversal of persistent tissue injury with decreased levels of pro-inflammatory cytokines; and (vi) CD11c+ cells transferred to liposome clodronate-treated mice were found to be in kidney interstitium in addition to liver, spleen and lungs.

The pathogenesis of ischaemic AKI involves multiple cellular, biochemical and molecular alterations resulting from a decrease in renal blood flow [2]. Endothelial cell injury is thought to play an important role in the initiation phase of AKI, and subsequent inflammation and tubular cell injury extend the initial injury, resulting in a decrease in the glomerular filtration rate [19]. Inflammation in the early reperfusion period is characterized by antigen-independent activation of neutrophils, macrophages, T cells or NKT cells [3] and participates in kidney injury by releasing a variety of mediators, such as inflammatory cytokines, chemokines and reactive oxygen species [8]. In addition to these cell types, a recent report has demonstrated that the resident CD11c+ DCs are a predominant source of pro-inflammatory cytokines and chemokines [12]. In contrast, at 20 h after liver I/R, liver DCs showed mature phenotype with increased production of IL-10 and TGF-β, suggesting an independent role of DCs in tissue injury or recovery processes by producing multiple cytokines [13]. However, macrophages or DCs are also well-known for their phenotypic heterogeneity, and this characteristic could lead us to hypothesize that DCs or macrophages might have a different role in late reperfusion or the recovery phase following IRI.

In this study, we first examined the phenotype of kidney CD11c+ dendritic subset at different time points following IRI and found that the percentage of CD11c+ cells that co-expressed surface activation markers increased at 72 h after reperfusion, indicating that kidney dendritic subset obtained a mature phenotype following IRI. This could also suggest that kidney dendritic subset in the late reperfusion or recovery phase has a different function compared to those cells in the early reperfusion phase that are known to secrete multiple pro-inflammatory cytokines, participating in kidney injury.

The intravenous injection of liposome clodronate results in systemic depletion of mononuclear cells, and recent reports have demonstrated a beneficial effect of systemic mononuclear cell depletion in a rodent model of IRI [16,17]. However, because we focused on the role of kidney DC subset in the late reperfusion or recovery phase, the first injection of liposome clodronate began 24 h after reperfusion, the time when regeneration usually begins [20]. Late administration of liposome clodronate resulted in more preferential depletion of kidney CD11+ F4/80+ dendritic subset rather than CD11c- F4/80+ macrophages, and the depletion of these cells was accompanied by persistent kidney tubular damage and inflammation. Tubular cell damage, as well as the number of apoptotic cells, increased significantly, and we also observed that cell proliferation following injury was also significantly impaired in dendritic subset-depleted kidneys, suggesting the important role of these cells in tubular regeneration process. In addition, observing same injury phenotype in Balb/c mice made us conclude that this finding of impaired recovery in dendritic subset-depleted status is not confined to one mouse strain. Cytokine profiles measured by CBA also
Fig. 7. Effect of CD11c$^+$ F4/80$^+$ cells on inflammation in recovery phase after IRI. (A) Kidney tissues from I/R + PBS and I/R + liposome clodronate-treated animals were obtained, and tissue levels of INF-γ, IL-6, TNF-α and IL-10 were measured by CBA and expressed as mean ± SE. *P < 0.05 compared with PBS. n = 4–5 per group. (B) Cell suspensions from sham, I/R + PBS and I/R + liposome clodronate-treated kidneys at I/R day 7 were stained with CD45 and Ly6G. The number of Ly6G$^+$ among CD45$^+$ cells was expressed as mean ± SE. *P < 0.05 compared to sham. n = 4–5 per group.
showed that dendritic subset depletion was associated with persistently increased pro-inflammatory cytokines, TNF-α, IL-6 and IFN-γ, in dendritic subset-depleted kidneys, while the anti-inflammatory cytokine IL-10 level showed the opposite result. These pro-inflammatory cytokines are well-known for a capacity to induce tissue injury and inflammation. Several studies have provided firm evidence that IFN-γ mediates IRI [21–24], and despite controversies about the role of IL-6, a recent study suggested that IL-6 is also maladaptive and contributes to ischaemic AKI [25]. Additionally, tissue inflammation, as assessed by kidney neutrophil counts that are known to aggravate tissue injury, was also increased in dendritic subset-depleted kidneys. All these results suggest that kidney CD11c(F4/80)+ dendritic subset in the late reperfusion phase could partially mediate recovery or regeneration following kidney IRI by modulating cytokine secretion. Both the protective role of DCs in the recovery phase and the pathogenetic role in the early phase in different kinds of injury models have been reported. In asthma models, pulmonary DCs capture airborne allergens and ameliorate disease by inducing CD4+ T regulatory 1-like cells that secrete IL-10 [15]. In nephrotoxic nephritis, DCs can also stimulate IL-10 production, contributing to protection by inducing regulatory T cells [14]. In addition, DCs freshly isolated from the liver after IRI exhibited a mature phenotype with an inhibitory profile of increased IL-10 [13]. In contrast, DCs could also participate in tissue injury by secreting pro-inflammatory cytokines [12]. Therefore, DCs are thought to have different phenotypes with secretion of a different profile of cytokines under different tissue milieu. Although our results positively suggest an important role of kidney dendritic subset in the recovery process following IRI, the results did not provide a definite answer for the functional role of these cells. To gain a better insight about the role of dendritic subset in the recovery process, we isolated kidney CD11c+ cells at different time points after reperfusion and measured cytokine and chemokine secretion. CD11c+ cells from the recovery phase (day 7) secreted significantly higher levels of IL-10, while the levels of TNF-α and IL-6 were not different compared to CD11c+ cells recovered in the early reperfusion phase. IL-10 is a potent negative regulator of immunoproliferative and inflammatory responses and also has beneficial effects on immune-mediated kidney disease. However, because the purity of isolated kidney-derived CD11c+ cells was only 90%, there is possibility that other contaminated cell types might have an effect on cytokine profile. Flow cytometric analysis using intracellular cytokine staining using kidney CD11c+ cells might help elucidate the role of these cell types more clearly. Although these results strongly suggest the independent participation of kidney CD11c+ dendritic subset by producing cytokines, there is also a possibility that these cells participate in tissue recovery by an interaction with Tregs. Recently, the role of CD4+ CD25+ FoxP3+ regulatory T cells in recovery phase after I/R has been demonstrated by Gandolfo et al. [26].

To investigate the direct causal relationship between kidney dendritic subset and tissue recovery, CD11c+ splenic DCs were injected after depletion. Adoptive transfer of CD11c+ splenic DCs after DCs depletion showed decreased tissue injury and levels of pro-inflammatory cytokines. Transferred cells were identified in perfused kidneys as well as liver, spleen and lung, confirming the transmigration of those infused CD11c+ cells into kidney tissue. However, the mechanism of beneficial effect of CD11c+ adoptive transfer is not clear. Both endocrine and paracrine effect of those infused cells might participate in recovery process of IRI.

However, these observations could firmly provide evidence that kidney CD11c(F4/80)+ dendritic subset in the recovery phase has an anti-inflammatory phenotype and directly participates in kidney recovery by modulating the inflammatory response.
Adoptive transfer of CD11c⁺ splenic DCs results in partial reversal of persistent tissue injury and also decreased level of pro-inflammatory cytokines. Mice were intravenously injected with either PBS or CD11c⁺ splenic DCs on day 5. CD11c⁺ cells from spleen were separated using anti-CD11c-coated microbeads and magnetic separation. Restaining of the separated cells for CD11c demonstrated clear enrichment for CD11c⁺ (90.6%) (A). Representative pictures of PAS-stained section of kidneys from mouse injected with PBS (B) or CD11c⁺ splenic DCs (C) (PAS, ×200). Semi-quantitative scoring of histologic injury (D). Kidney tissues from I/R + liposome clodronate + PBS and I/R + liposome clodronate + CD11c⁺ adoptively transferred animals were obtained, and CBA was done to measure tissue cytokine levels (E and F). *P < 0.05 compared with I/R + liposome clodronate + PBS. n = 4–5 per group.
In conclusion, we have demonstrated a beneficial effect of kidney CD11c$^+$ F4/80$^+$ dendritic subset in the recovery process from IRI by modulating inflammatory responses. Insight into the mechanisms regarding recovery or regeneration could help develop various targets and tools that ultimately could enhance patient prognosis from AKI.

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Conflict of interest statement. None declared.

References


Fig. 10. In vivo tracking of adoptively transferred CD11c$^+$ cells. Mice were intravenously injected with CellTracker$^{TM}$ CM-Dil-labelled CD11c$^+$ cells following liposome clodronate injection after I/R. At day 7, both kidneys, lungs, liver and spleen were perfusion fixed, and fluorescence microscopy was applied to frozen sections for tracking infused CD11c$^+$ cells. Representative pictures of kidneys infused with Dil-labelled CD11c$^+$ cells after liposome clodronate injection. Dil-positive transferred CD11c$^+$ cells were occasionally identified juxtaposition to tubular epithelial cells (A). Picture of kidney transferred with non-labelled CD11c$^+$ cells (B). Dil-positive cells were also identified in spleen, liver and lung (C, D, E).
Augmenter of liver regeneration protects kidneys from I/R injury

Xiao-hui Liao\(^1\), Ling Zhang\(^1\), Qi Liu\(^2\), Hang Sun\(^2\), Chao-ming Peng\(^1\) and Hui Guo\(^2\)

\(^1\)Department of Nephrology, The Second Affiliated Hospital, Chongqing Medical University, Chongqing 400010, China and 
\(^2\)Institute for Viral Hepatitis, Key Laboratory of Molecular Biology for Infectious Diseases, The Second Affiliated Hospital, Chongqing Medical University, Chongqing 400010, China

Correspondence and offprint requests to: Ling Zhang; E-mail: lindazhang8508@hotmail.com.

Abstract

Background. Augmenter of liver regeneration (ALR), which was identified originally for its crucial role in promoting hepatocyte proliferation, is expressed in both the liver and kidney. Protective effects of ALR have been demonstrated in experimental models of acute liver failure. In the present study, we investigated the effect of ALR on renal ischaemia/reperfusion (I/R) injury and the possible mechanisms of its action.

Methods. Male Sprague–Dawley rats were subjected to renal ischaemia for 60 min and then administered with either saline or recombinant human ALR (rhALR). A sham-operated group served as control. The expression of ALR in the sham-operated and acute kidney injury (AKI) groups was detected by immunohistochemistry and western blotting. Renal dysfunction and injury were assessed by measurement of serum biochemical markers and histological grading. Expression of prolif-