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Interleukin-33 signaling contributes to renal fibrosis following ischemia reperfusion

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ABSTRACT

Acute kidney injury caused by ischemia-reperfusion injury (IRI) is a major risk factor for chronic kidney disease, which is characterized by renal interstitial fibrosis. However, the molecular mechanisms underlying renal fibrosis induced by IRI are not fully understood. Our results showed that interleukin (IL)–33 was induced markedly after IRI insult, and the kidneys of mice following IRI plus IL-33 treatment presented more severe renal fibrosis compared with mice treated with IRI alone. Therefore, we investigated whether inhibition of IL-33 protects against IRI-induced renal fibrosis. Mice were administrated with soluble ST2 (sST2), a decoy receptor that neutralizes IL-33 activity, or vehicle by intraperitoneal injection for 14 days after IRI challenge. We revealed that mice treated with sST2 exhibited less severe renal dysfunction and fibrosis in response to IRI compared with vehicle-treated mice. Inhibition of IL-33 suppressed bone marrow–derived fibroblast accumulation and myofibroblast formation in the kidneys after IRI stress, which was associated with less expression of extracellular matrix proteins. Furthermore, inhibition of IL-33 also showed a significant reduction of $F4/80^+$ macrophages and $CD3^+$ T cells in the kidneys of mice after IRI treatment. Finally, Treatment with IL-33 inhibitor reduced proinflammatory cytokine and chemokine levels in the kidneys of mice following IRI insult. Taken together, our findings indicate that IL-33 signaling plays a critical role in the pathogenesis of IRI–induced renal fibrosis through regulating myeloid fibroblast accumulation, inflammation cell infiltration, and the expression of proinflammatory cytokines and chemokines.

1. Introduction

Acute kidney injury (AKI) is defined as an abrupt deterioration of renal function after exposure to various insults such as major cardiovascular surgery, abdominal organ transplantation, and nephrotoxic drugs (Smoyer et al., 2016; Goren and Matot, 2015). It is well documented that renal ischemia-reperfusion injury (IRI) remains one of the leading causes of AKI (Fukazawa and Lee, 2014). Patients recovering from AKI have a significant risk of progression to chronic kidney disease (CKD) or end stage renal diseases , which is manifested by progressive development of interstitial fibrosis (Heung and Chawla, 2014). Currently, therapeutic options for this severe complication are very limited except for dialysis or kidney replacement (Zhou and Liu, 2016). Therefore, an improved understanding of the pathogenesis regarding IRI-induced renal fibrosis is critical for the development of therapies to prevent this devastating disease.

Interleukin (IL)–33 has been recently identified as a new member of the IL-1 cytokine family and a critical regulator of inflammatory and immune processes (Vocca et al., 2015; Reichenbach et al., 2015). It is reported that IL-33/ST2 signaling strongly promoted pulmonary fibrosis (Li et al., 2014). In addition, a recent work has demonstrated that IL-33 exacerbates cisplatin-induced AKI (Akcay et al., 2011). Chen et al. have revealed that IL-33 accentuates renal fibrosis in a unilateral urinary obstruction model (Chen et al., 2016). However, the role of IL-33 in IRI-induced renal fibrosis and related mechanism remains unknown. In the current study, our findings showed that IL-33 is upregulated in the kidney in response to IRI-induced renal injury and fibrosis, and IL-33 treatment potentiates IRI-induced renal fibrosis. Thus, we examined whether inhibition of IL-33 attenuates IRI-induced renal fibrosis. Using soluble ST2 (sST2), a decoy receptor that is able to neutralize IL-33 biological activity, we showed that inhibition of IL-33 protects the kidney from IRI-induced renal injury and fibrosis through inhibiting bone marrow–derived fibroblast accumulation, macrophage and T cell infiltration, and the expressions of proinflammatory cytokines and chemokines.

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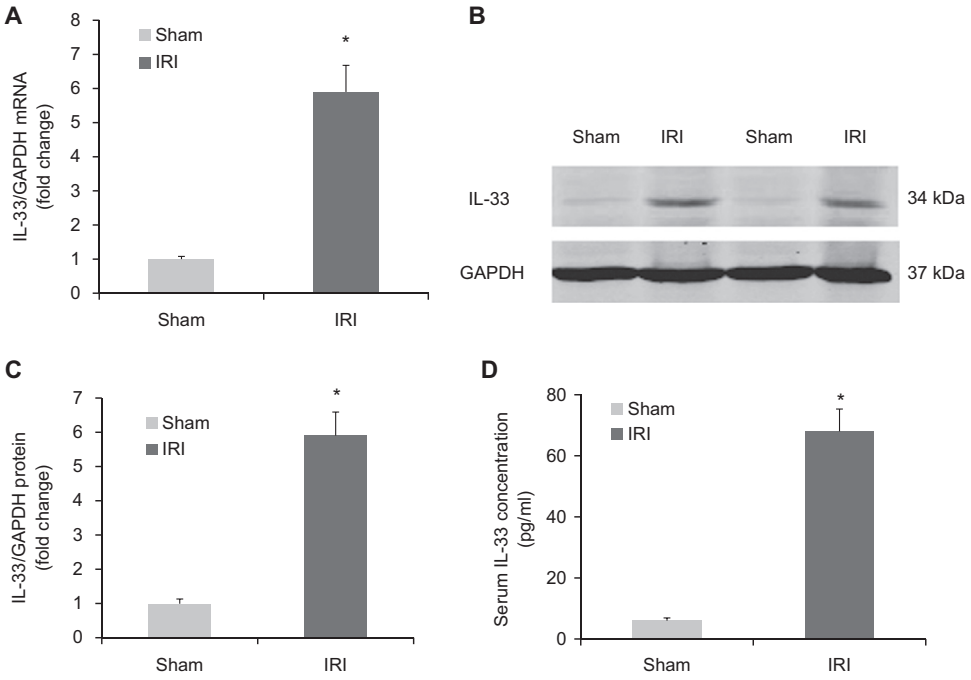

Fig. 1. IL-33 is induced in the kidney after IRI treatment. (A) IL-33 mRNA is induced substantially in kidneys of mice at 14 days after IRI challenge compared with sham control mice. (B) Representative western blot shows IL-33 protein levels in the kidneys of mice at 14 days after sham or IRI treatment. (C) Quantitative analysis of IL-33 protein expression in the kidneys. (D) Serum IL-33 is elevated significantly in kidneys of mice after IRI treatment. *$P < 0.01$ vs. sham. n=6 in each group. IRI, ischemia-reperfusion injury. Scale bar: 50 μm.

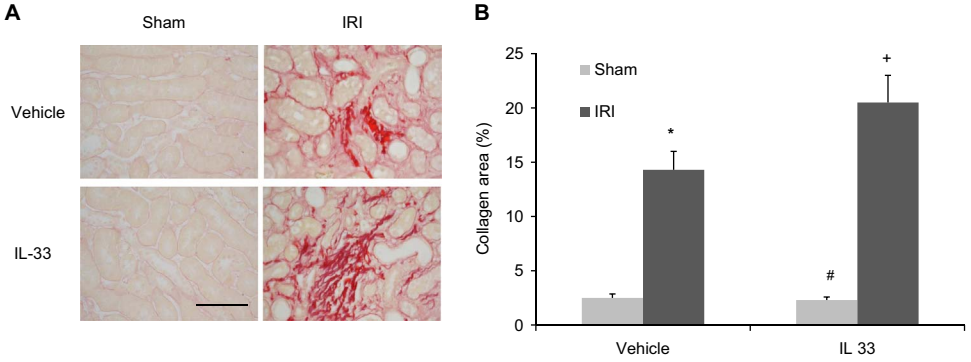

Fig. 2. IL-33 administration contributes to IRI-induced renal fibrosis. (A) Representative photomicrographs of kidney sections stained with Picrosirius red for evaluation of total collagen deposition in the kidneys at 2 weeks after sham or IRI treatment. (B) Quantitative analysis of renal interstitial collagen deposition in the kidneys. *$P < 0.05$ vs. sham. n=6 in each group. IRI, ischemia-reperfusion injury. Scale bar: 50 μm.

2. Material and methods

2.1. Animals and model of IRI-induced renal fibrosis

Male C57BL/6 mice (weighing 20–30 g, 8–10 weeks of age) were supplied by the Guangdong Province Laboratory Animal Center. Mice were housed in a specific pathogen-free environment at optimal temperature with a 12 h light/dark cycle. The mice were also provided free access to water and food ad libitum. Animal handling and surgical procedures were performed according to protocols approved by the Institutional Animal Care and Use Committee of the Sun Yat-Sen University. All efforts were made to minimize suffering. To induce the unilateral IRI-induced renal fibrosis model, mice were anesthetized with intraperitoneal injection ketamine. The right renal pedicle was bluntly dissected and exposed using a dorsal lumbotomy incision. A non-traumatic microaneurysm clamp was applied to the right renal artery for half hour unilateral clamping. Reperfusion was confirmed visually after clamps were removed. Nephrectomy of contralateral kidney was performed 5 d after unilateral IRI surgery, which allows evaluating renal function after unilateral IRI. Sham animals were subjected to the same surgical procedure except the renal pedicle was not clamped. Mice were placed on a heating pad to maintain body temperature at 37 °C during surgery. All mice were killed on day 14 after IRI or sham surgery. Systemic perfusion of mice was conducted with PBS, and kidneys were then rapidly harvested.

2.2. ELISA

IL-33 levels in the serum were measured by ELISA using the Mouse IL-33 ELISA kit (Thermo Fisher) according to the manufacturer's instructions. Blood urea nitrogen was determined to assess renal function by using a Quantichrom assay kit (BioAssay Systems, Hayward, CA) according to the manufacturer's protocol. ELISA kits (R & D Systems) were utilized to quantify IL-6, transforming growth factor (TGF)-β1, monocyte chemotactic protein (MCP)–1, and CXC chemokine ligand (CXCL) 16 in plasma according to the manufacturer's instructions.