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IL-37b protects against renal ischemic/reperfusion injury via inhibition NF-κB up-regulation

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Abstract

IL-37 member suppresses inflammation among the various splicing forms of IL-37, IL-37b with unclear signaling pathway. Acute kidney injury resulted from ischemia and reperfusion is a significant clinical problem in cardiovascular medical and surgical procedures. In this study, we investigated the effects of IL-37b renal I/R injury in mice and to determine the involvement of nuclear factor kappa B (NF-κB) activation in the effects. Renal I/R was induced by right renal pedicle clamping for 45 min and left nephrectomy. Sham-operated mice with no occlusion of the renal vessel. I/R mice received an injection of recombinant IL-37b or vehicle, immediately before reperfusion. Compared with vehicle treatment, mice treated with recombinant IL-37b attenuates I/R-induced TNFα, IL-1β, and IL-6, inhibited the up-regulation of NF-κB activation after I/R with decreased the levels of plasma of both creatinine and urea. In conclusion; IL-37b exerts effects in vivo by modulating the NF-κB activation.

Keywords: IL-37; Renal I/R; Cardiovascular diseases; Recombinant IL-37b

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Introduction

Inflammatory response is now believed to play a central role in the pathophysiology of renal ischemia and reperfusion injury (I/R) [1]. The events of I/R-induced inflammatory responses modulate the inflammatory cells move from the blood stream and attract to the renal tissues [2]. Furthermore, the I/R-injury is a leading cause of acute renal failure and it is a common renal problem that is still associated with high mortality, despite significant advances in the management regimen [3]. I/R is caused by a suddenly transient drop in blood flow associated with a robust inflammatory and oxidative stress response to hypoxia and reperfusion, frequently occurring during shock, sepsis and transplantation [4]. Although important findings have been made in

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the definition of the cell biologic consequences of I/R to investigate therapies for this clinical problem [5]. IL-37 (formerly named IL-1F7) is the most recently identified member of the IL-1 family [6]. Currently, IL-37 has been identified only in certain types of human cells, and a mouse homolog has not been identified. IL-37b is the best characterized isoform of the various splice variants of IL-37 [7]. IL-37b exhibits potent immunosuppressive functions against both innate and adaptive immunity. The expression of IL-37b in macrophages or epithelial cells almost completely suppresses the production of proinflammatory cytokine [8]. Mice that transgenically express human IL-37b are protected from lipopolysaccharide (LPS) induced septic shock and dextran sulfate sodium-induced colitis through the inhibition of DCs activation and the downregulation of circulating and tissue cytokines [9, 10,11]. In addition, there is evidence indicating a critical role of Nuclear factor-κB (NF-κB) in the pathogenesis of renal I/R injury. Activation of NF-κB has a major role in the pathophysiology of experimental ischemic I/R injury [12, 13, 14].

NF-κB is the best-known mediator of TNF-α and IL-6-associated cellular response. In the present study, we adopted the mice model of renal I/R injury to test the hypothesis that IL-37b attenuates inflammatory response after renal I/R by suppressing NF-κB activity. Many published articles consider the critical role of cytokines in regulatory proteins or glycoproteins released from various cells, mainly from leukocytes, usually by various activating stimuli, and regulate the development and effector functions of immune cells. Most cytokines show autocrine and/or paracrine action and a few of them exhibit endocrine action. Ischemia reperfusion-induced acute kidney injury causes the synthesis of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α [15].

Materials and methods

Animals

Male C57BL/6 mice aged 8–10 weeks (25-28 g), were purchased from the Jackson Laboratory. The mice received a standard diet and water ad libitum at the Eastern Virginia Medical School Animal Care Facility according to institutional and all procedures were previously reviewed and approved by the Ethics Committee of the Eastern Virginia Medical School, Norfolk, VA (document number 1223/2015).

Surgical model of renal I/R

The study design divided the mice into the following groups (n = 8 per group): sham group), I/R injury group, mice pretreated with IL-37b at dose of 2 μg/kg (Adipogen AG, Liestal, Switzerland) (dissolved in or 200 μl normal saline (vehicle group), which were administered via the tail vein immediately before reperfusion in I/R mice. Surgery was performed as previously described [16, 17]. Briefly, mice were anesthetized with Ketamine-Xylazine, a midline incision was made and right renal pedicle was cross-clamped for 45 minutes and left nephrectomy. During the procedure,

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animals were kept well hydrated with saline and at a constant temperature (37°C) through a heating pad device. 45 min after ischemia, microsurgery clamps were removed. The mice were sacrificed after 48 hours of reperfusion.

Enzyme-linked immunosorbent assay

The kidney tissue was homogenized, centrifuged for 10 min at 12 000 g and 4°C, and the supernatants were collected and stored at −80°C. The levels of IL-6, IL-18 and TNF-α in kidney tissue and serum were measured using special commercial ELISA kits (R&D Systems). All tests on samples and standards were performed in duplicate.

Analysis of Renal Function

Serum creatinine was used for renal function assessment. Blood samples were collected at 4, 24, and 48 hours post reperfusion from the abdominal inferior cava vein immediately before induced death. Serum samples were analyzed on a Cobas Mira Plus (Roche, Mannhein, Germany), using the modified Jaffé technique.

Western blot analysis

The kidney sample was homogenized in whole cell lysis buffer (Cell Signaling Technology, Danvers, MA, USA) that contained additional phosphatase and protease inhibitors as described previously [18]. Briefly, the kidney tissue homogenate was then centrifuged at 18 000 g for 30 min at 4°C. The protein concentration was measured in the supernatants using the Bradford assay (Bio-Rad Laboratories, Hercules, CA, USA). The samples were subjected to sodium dodecyl sulphatepolyacryl-amide gel electrophoresis (SDS-PAGE). The proteins were transferred onto poly-vinylidene fluoride microporous mem-branes (Bio-Rad) and probed with primary antibodies. The antibodies used in this study included anti-NF-κB (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA CA), and anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH). An Invitrogen kit (Invitrogen, Carlsbad, CA, USA) was used to visualize the protein bands. Densitometry was performed using the Image Pro Plus software (Media Cybernetics, Silver Spring, MD, USA). To standardize the densitometry measurements between individual samples, the ratios of NF-κB to GAPDH were calculated for statistical analyses.

Myeloperoxidase (MPO) activity

Myeloperoxidase (MPO) activity. MPO activity, used as an indicator of neutrophil infiltration, was measured (18) at 4, 24, 48. Briefly, aliquots (0.2 ml) of 40,000g supernatants of kidney homogenates were added to 0.8 ml of reaction buffer containing 50 mM potassium phosphate, pH 6.0, 0.2 mg/ml O-dianisidine dihydrochloride, and 0.0006% H_2O_2. Absorbance was determined at 460 nm. MPO activity, normalized to protein content of the supernatant, was expressed as the percentage of measured activity in kidneys from mice subjected to sham operations.

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Statistical Analysis

All data were described as mean±SEM. Different results among groups were compared using Kruskal-Wallis One Way Analysis of Variance on Ranks (ANOVA) or by the T-test. Results were considered significant when $P<0.05$. Survival curves were estimated by the Kaplan-Meier test. All statistical analyses were performed with the aid of SigmaStat Statistical Software 2.0 (Jandel Corpomiceion, TX, USA).

Results

Improve of renal function by IL-37b

All animals subjected to renal I/R injury showed a significant rise in serum creatinine levels (sCr) after 6, 24 and 48 hours of reperfusion, in comparison to sham-operated, IL-37b had a significant reduction in sCr at all times analyzed, sCr levels were unaffected in untreated versus treated animals), **Fig. 1**.

IL-37 protects against renal I/R injury

In order to confirm the protective effect of IL-37b, the change of renal pathological morphology was analyzed in the mice following I/R model according to the Hamar score method [19]. The results in **Fig. 2** indicated that in the Sham group, normal kidney tissues were without lumen expansion and epithelial cell flattening in the glomerular and renal tubule; however, when mice were exposed to I/R injury, kidney pathological damage was clear: Renal tubular epithelial cell degeneration, renal tubular cavity expansion and tube formation and renal interstitial inflammatory cell infiltration are all serious. IL-37b treatment protected against I/R-induced renal damage.

IL-37b ameliorates the inflammatory reaction of kidney

Renal I/R injury was always associated with inflammatory reactions. Thus, in order to explore the protective mechanisms of I/R for renal injury, representative inflammatory factors, such as IL-6, IL-18 and TNF-α were detected. **Fig. 3** demonstrates that IL-6, IL-18 and TNF-α were all significantly increased in the I/R group and IL-37b pretreatment downregulated the increase of these three types of inflammatory factors. These results suggested that the protective function of IL-37b was associated with its anti-inflammatory effects.

IL-37b suppresses NF-κB following I/R injury in mice

I/R renal injury signaling activation-induced innate immune response plays an important role in renal I/R injury. **Fig. 4** shows that NF-κB activation was suppressed significantly in the IL-37-treated group compared with the untreated group which is consistent with the results of the reduced production of inflammatory cytokines.

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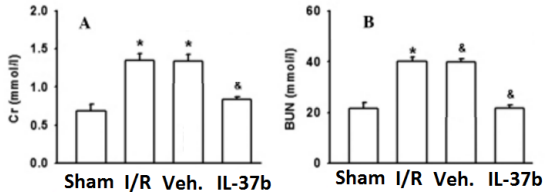

Figure 1.

Renal I/R-induces damage to the renal function, which was followed by treatment with IL-37b in the mice. The levels of (A) creatinine (Cr) and (B) blood urea nitrogen (BUN) when mice were exposed to I/R, IL-37b or Vehicle. IL-37b (2 μg/kg) was pretreated before I/R. (*n*=8 in each group). *P<0.05 vs. Sham group; #P<0.05 vs. IL37b group.

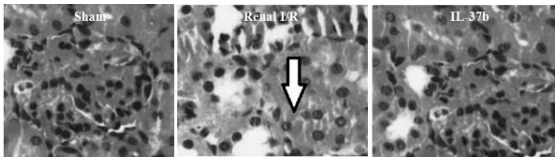

Figure 2.

Renal I/R-induces damage to the renal tissue, which was followed by treatment with IL-37b in mice. Histopathological changes in the kidney tissue (hematoxylin and eosin staining; original magnification, x200). I/R injury to generate the mice ischemia model. IL-37b (2 μg/kg) was pretreated immediately before I/R. Arrow: Dilatant renal tubules and renal interstitial infiltration of the inflammatory cells to I/R mice.

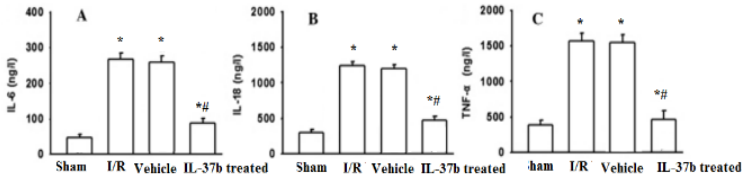

Figure 3.

Renal I/R induces changes of IL-6, IL-18 or TNF-α, followed by treatment with IL-37b in the mice. The changes of (A) IL-6, (B) IL-18 and (C) TNF-α when mice were exposed to I/R, IL37b or Vehicle. IL-37b was injected in the tail vein of the mice I/R models. (*n*=8 in each group). *P<0.05 vs. Sham group; #P<0.05 vs. I/R group.

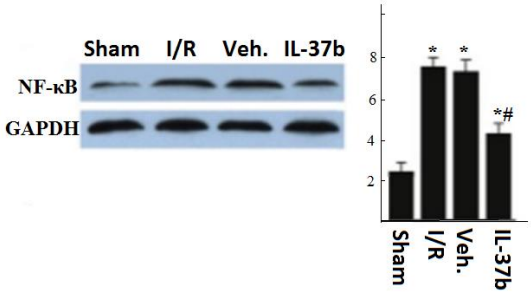

Figure 4.

IL-37 suppresses NF-κB activation and further, induces anti-inflammatory cytokine expression in mice renal I/R injury. Hearts were retrieved Quantitative densitometric analysis NF-κB with GAPDH as an internal standard ($n = 6$). $^{*}P < 0{\cdot}05$; $^{*\#}P < 0{\cdot}05$.

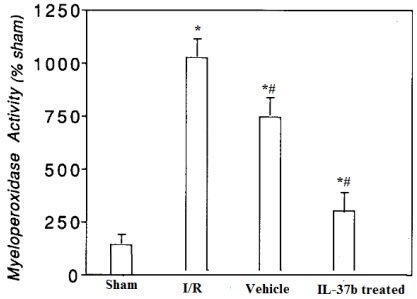

Figure 5.

Effect of renal ischemia on MPO activity in Renal I/R injury mice. MPO activity was measured in kidney tissue 4, 24, 48 after reperfusion, vehicle or sham surgery. P values are presented for comparison between sham and IL-37b treated mice.

Discussion

The present study is to examine the role of IL-37 in mouse renal I/R injury. Our data demonstrated that IL-37b pre-treatment markedly ameliorated I/R injury, as demonstrated by reduced the levels of creatinine (Cr) and blood urea nitrogen, and improved renal function. Regarding the immunological aspect of renal I/R injury, T lymphocytes are considered important mediators of this

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injury, since T cell depletion significantly improves renal function [20]. In this context, studies [21, 22], point out that tissue outcome after renal I/R injury may be influenced by anti and pro inflammatory responses. CD4 T cells have been a focus of research due to their differentiation to either a Th1 (pro inflammatory) or a counter-balancing Th2 (anti-inflammatory) pro-file.

In renal I/R injury mice lacking the transcriptional factor of the Th2 profile (STAT-6) develop more severe renal damage, while deletion of the Th1 transcriptional factor STAT-4 results in mild protection. Furthermore, activation of the Th2 transcriptional factor GATA-3 followed by IL-4, IL-5, IL-6, IL-10 and IL-13 production was shown to be protective, whereas higher expression of the transcriptional factor T-bet, and IL-12, IL-1β and IFN-γ was deleterious [23]. Furthermore, it has recently been reported that in addition to a nuclear function, IL-37b acts as an extracellular cytokine by binding to its receptor complex, IL-1R8–IL-18Rα, to activate a multifaceted intracellular anti-inflammatory program [24]. The mRNA expression of IL-18Rα was quite low in Tregs and MDSCs compared with $CD4^+$ $CD25^-$ T cells, suggesting that IL-37b may primarily indirectly, not directly, promote the differentiation of Tregs and MDSCs. IL-37 undoubtedly functions as a promising beneficial compound to restrain excessive inflammatory responses [25]. In this study, we found that IL-37 can suppress NF-κB signaling activation after I/R injury. Studies have demonstrated that mature IL-37 translocated to the nucleus via caspase-1 and binded to phosphorylated Smad3 to form an IL-37/Smad3 complex in the perinuclear region, which affected pro-inflammatory gene transcription [26]. Indeed, Smad3 deficiency has been shown to result in the hyperactivation of innate immune cells, which occurs in response to TLR signaling and subsequent NF-κB activation [27].

Taken together, our results showed that the protection found under IL-37b involves a shift towards down regulation of pro-inflammatory molecules and upregulation of anti-inflammatory ones. Since IL-37b proved to be protective, we believe that this may represent a new therapeutic strategy against renal I/R injury

Competing interests

Authors declare that we have no competing interests.

Authors Contribution

Yan Xina, and Howard D. Kirshnera conducted the experiment; Carmen A. Peralta and Richard A. Ciavarra designed the study; Howard D. Kirshnera, Carmen A. Peralta and analyzed the data; and all authors shared in wrote the paper.

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