Int J Clin Exp Pathol 2017;10(11):10998-11005
www.ijcep.com /ISSN:1936-2625/IJCEP0061765

Original Article

The renal level of a novel cytokine IL-35 is related to sepsis-associated acute kidney injury in mice

Fenghua Yao[1,2*], Li Zhang[1*], Zhong Yin[1], Bo Fu[1], Zhe Feng[1], Zongze He[1], Qinggang Li[1], Jijun Li[2], Xiangmei Chen[1]

[1]*Department of Nephrology, Chinese PLA General Hospital, Chinese PLA Institute of Nephrology, State Key Laboratory of Kidney Diseases, National Clinical Research Center for Kidney Diseases, Beijing, China;* [2]*First Hospital Affiliated to The Chinese PLA General Hospital, Beijing, China.* [*]*Equal contributors.*

Received July 18, 2017; Accepted August 28, 2017; Epub November 1, 2017; Published November 15, 2017

Abstract: Interleukin-35 (IL-35) is a novel immunosuppressive and anti-inflammatory cytokine. IL-35 is mainly secreted by regulatory T cells (Tregs), and exerts its effects through inducing proliferation of Tregs and reducing activity of helper T cells Th17. However, the effect of IL-35 on sepsis-associated acute kidney injury (SA-AKI) remains unclear. This study is aimed to examine the expression and role of IL-35 in an animal model of SA-AKI induced by cecal ligation and puncture (CLP). Eleven C57 male mice with SA-AKI and eight controls were used, and blood and kidney tissues were collected. Blood creatinine (Cr), urea nitrogen (BUN), alanine transaminase (ALT) and aspartate transaminase (AST) were measured to assess kidney and liver injury. The renal morphology and cell apoptosis were examined. The mRNA and protein expression levels of IL-35 in kidney tissues were tested by qRT-PCR, IHC-P, IF, and ELISA. Biochemical and histological examinations indicated CLP induced SA-AKI in mice. TUNEL assay showed apoptosis of renal tubular epithelial cells in SA-AKI mice. The mRNA and protein expression of IL-12α and EBI3 in kidney tissues decreased significantly in SA-AKI mice compared with those in sham controls. IL-35 levels in kidney tissues displayed a significantly negative correlation with the levels of Cr (r = -0.584, P = 0.009), ALT (r = -0.549, P = 0.015), AST (r = -0.475, P = 0.04), but not with BUN (r = -0.437, P = 0.061). These results demonstrated that IL-35 is associated with the pathological process of SA-AKI, and might represent a potential therapeutic agent for SA-AKI treatment.

Keywords: Sepsis, acute kidney injury, interleukin-35, regulatory T cells

Introduction

Sepsis is believed to be the most common cause leading to acute kidney injury (AKI) in critically ill patients. Although sepsis is an independent risk factor associated with deteriorative outcomes [1], the mechanisms underlying AKI development in septic patients remain unclear. We suspect that interleukin-35 (IL-35) is a cytokine which may contribute to the development and progress of AKI.

IL-35 is a novel member of interleukin-12 (IL-12) family with anti-inflammatory and immunosuppressive properties. It is composed of two subunits IL-27β and interleukin-12 alpha (IL-12α), which are encoded by two genes: Epstein-Barr virus-induced gene 3 (EBI3) and IL-12α. IL-35 is secreted by regulatory T-cells (Treg) and suppresses inflammatory immune cells. Eectopic expression of IL-35 was found to regulate naive T cells, while recombinant IL-35 was shown to suppress the proliferation of T-cell [2].

IL-35 is found to be highly expressed on stimulated human and non-stimulated mouse Treg cells, but undetectable in non-stimulated human Treg cells [3]. Moreover, IL-35 was demonstrated to be increased in human non-T cells including aortic smooth muscle cells and microvascular endothelial cells, after tumor necrosis factor (TNF-α), interferon-γ (IFN-γ) and IL-1β stimulations [4]. In vivo, IL-35 showed suppressive effects on arthritis induced by collagen in mice by enhancing IFN-γ synthesis and decreasing IL-17 production [5, 6]. In addition, IL-35 attenuated effectively airway inflammation induced by allergen-specific CD4+ Th2 cells [7]. IL-35 produced by inducible costimulator (ICOS)-positive Treg cells was found to reverse allergic airways disease in mice [8].

So far, very little evidence has been published on the function of IL-35 in kidney injury. Only one report indicated the lower expression of IL-35 in diabetes-induced renal damage [9]. In the present study, a cecal ligation and puncture (CLP) method was used to generate sepsis-associated acute kidney injury (SA-AKI) in mice and further examined the pathophysiology of SA-AKI. We hypothesized that immunosuppressive and anti-inflammatory cytokine IL-35 is related to the development and progress of SA-AKI. To our knowledge, this is the first study to explore the possible functions of IL-35 in the pathogenesis of SA-AKI.

Materials and methods

Animals

6-8 weeks old male C57BL/6 mice (weighing 20-25 g) were purchased from and kept at the Animal Center of General Hospital of The Chinese People's Liberation Army (PLA) (Beijing, China). The mice were maintained in a 12-h light/dark cycle at 25°C on a chow diet, in accordance with the institutional guidelines. The animal study was approved by the Ethics Committee from Chinese PLA General Hospital.

SA-AKI model induced by cecal ligation and puncture

After being acclimated for 1 week before the experiments, mice were anesthetized by intraperitoneal injection of 2% pentobarbital. After a middle abdominal incision, the cecum was delivered, ligated with a silk suture at the position of 1 cm from the tip, and punctured doubly using a 21-gauge needle. The cecum was squeezed gently to extrude a small amount of feces, and then put back to the abdominal cavity. The incision was then closed. Control mice received sham operation, in terms of the same laparotomy without cecum ligation and puncture. Eleven mice were employed for SA-AKI models, and eight were used as sham controls. Mouse serum samples and the kidneys were harvested 24 h after surgery.

Biochemical and histological examination

Blood urea nitrogen (BUN), serum creatinine (Cr), alanine transaminase (ALT) and aspartate transaminase (AST) were measured to assess kidney and liver injury using a Hitachi 747 automatic analyzer (Hitachi, Tokyo, Japan). For histopathological assessment of SA-AKI, kidney tissues were fixed in 10% formaldehyde for at least 24 h. Specimens were embedded in paraffin, and the midsagittal sections at 4-μm thickness were subjected to periodic acid-Schiff (PAS) staining and TUNEL assay (Roche, USA) for tubular injury and apoptosis assessment according to the manufacturer's protocol. Sections were observed under an optic microscope (Nikon, Tokyo, Japan) at a 400× magnification and evaluated by three independent pathologists. The number of TUNEL-positive cells per high power field was counted in the renal cortex and outer medulla in a blinded fashion.

RNA extraction and quantitative real-time polymerase chain reaction (RT-PCR)

For gene expression analysis, total mRNA was isolated from kidney tissue using TRIzol reagent (Invitrogen, USA) according to the manufacturer's instructions. 1 μg of total RNA was reverse-transcribed into cDNA using the First-strand cDNA synthesis super Mix (Applied Biosystems, USA) based on the manufacturer's instructions. IL-35 gene expression was measured by real-time PCR performed in 20 μL volume containing 0.2 μL of cDNA, 10 μL of Power SYBR Green PCR Master Mix (Biosystems, UK) and 1 μL of each primer (IL-12α: sense 5'-CACTCCCAAAACCTGCTGAG-3', antisense 5'-TCTCTTCAGAAG TGCAAGGGTA-3'; EBI3: sense 5'-GAAGTACTGGATCCGTTACAAGC-3', antisense 5'-GAAGGACGTGGCTTCAATG-3'; GAPDH: sense 5'-AGCCACATCGCTCAGACAC-3', antisense 5'-GCCCAATACGACCAAATCC-3'). PCR reactions were run for 40 cycles in a Light Cycler 2.0 (Roche Diagnostics, Germany). Each cycle consisted of 10 min 95°C denaturation, 30 s 95°C primer annealing and extension for 30 s at 60°C and 30 s at 72°C. Each sample was tested in triplicate. The data was analyzed using the $2^{-\Delta\Delta Ct}$ method. All samples were normalized to an endogenous control GAPDH.

Immunohistochemistry assay

Sections of 4 μm thick from formalin-fixed and paraffin-embedded tissues were mounted on slides, followed by being deparaffinized and rehydrated. 3% H_2O_2 was used to block endogenous peroxidase for 20 min. 3% normal serum was employed as protein blocker for 30 min.

Table 1. Biochemical examination of the mice

	Sham (n = 8)	SA-AKI (n = 11)
Cr (μmol/L)	10.53 ± 2.18	32.34 ± 17.32*
BUN (mmol/L)	8.45 ± 1.02	25.48 ± 15.61*
ALT (U/L)	34.06 ± 13.20	132.80 ± 48.20*
AST (U/L)	114.41 ± 49.19	525.68 ± 384.84*

Data represent mean ± SEM. *P<0.05 versus sham. Cr-creatinine; BUN-blood urea nitrogen; ALT-alanine transaminase; AST-aspartate transaminase.

Sections were incubated with 1:500 anti-mouse EBI3 rat monoclonal antibody (Abcam, USA) and 1:2000 anti-mouse IL-12α rabbit monoclonal antibody (Abcam, USA) at 4°C for 18 h, followed by incubated with biotinylated goat anti-rat antibody (ABC Staining System, USA) and goat anti-rabbit antibody (Santa Cruz biotechnology, USA) at room temperature for 60 min. Slides were then incubated with horseradish peroxidase (HRP) streptavidin for 45 min, and peroxidase substrate 3,30-diaminobenzidine (DAB) (Sigma, USA) for 10 min. The sections were counterstained with hematoxylin, dehydrated with alcohol and xylene, and mounted in resin. Stained sections were observed under a light microscope (Nikon, Tokyo, Japan) at a magnification of 200× and evaluated by three independent pathologists.

Immunofluorescence

The expression of IL-35 in mouse renal tissues was confirmed by double immunofluorescence labeling. Briefly, the renal tissues were immediately fixed in liquid nitrogen after collection. 5 μm thick frozen sections were prepared. After antigen retrieval with 0.01 mol/L EDTA, tissue sections were incubated overnight at 4°C with 1:2000 rabbit monoclonal antibody against mouse IL-12α (Abcam, USA) and 1:200 rat monoclonal antibody raised against mouse EBI3 (Abcam, USA). After washing, tissue sections were incubated with secondary antibodies (1:400 dilution for Cy3-conjugated goat anti-rabbit IgG, and 1:50 dilution for FITC-conjugated donkey anti-rat IgG from Jackson ImmunoResearch, USA) for 1 h at 25°C in the dark. Cell nuclei were stained with 0.1 μg/ml DAPI (Abcam, USA). Finally, the slides were observed under a confocal laser scanning microscope (Olympus FluoView 1000) at a magnification of 400×.

Protein extraction and measurement of renal IL-35 level by enzyme-linked immunosorbent assay (ELISA)

Kidney lysates were prepared by homogenization on ice for 30 min using radioimmunoprecipitation assay buffer [50 mM Tris-Cl (pH 7.6), 150 mM NaCl, 1% NP-40, 0.1% sodium dodecyl sulfate, 0.5% deoxycholic acid, 1 μg/mL leupeptin, 1 μg/mL aprotinin, and 0.5 mM phenylmethylsulfonyl fluoride], and then centrifuged at 12,000 rpm for 30 min at 4°C, after which supernatants were collected. Renal IL-35 levels were measured using ELISA kits (BioLegend, San Diego, CA, USA) according to the manufacturer's instructions. The level of IL-35 was determined using Varioskan Flash (Thermo Scientific, Massachusetts, USA) at 450 nm absorbance. The minimal detectable levels were 0.12 ng/ml for IL-35. All samples were tested in duplicate, and the mean value was used for statistical analysis.

Statistical analysis

All data were shown as mean ± SEM. The differences between SA-AKI mice and the control groups were analyzed by unpaired Student's t test. Correlation analysis was carried out using Pearson's method. A P value<0.05 was considered as statistically significant.

Results

Biochemical and histological examination

Serum Cr, BUN, ALT and AST levels were measured to evaluate the development of SA-AKI (**Table 1**). Our results showed that CLP in mice induced polymicrobial peritonitis with multiple organ dysfunction, including AKI, indicated by the significant increase of Cr (32.34 ± 17.32 μmol/Lin CLP vs. 10.53 ± 2.18 μmol/L in sham) and BUN (25.48 ± 15.61 mmol/L in CLP vs. 8.45 ± 1.02 mmol/L in sham), as well as liver dysfunction indicated by the dramatically enhanced expression of ALT (132.80 ± 48.20 U/L in CLP vs. 34.06 ± 13.20 U/L in sham) and AST (525.68 ± 384.84 U/L in CLP vs. 114.41 ± 49.19 U/L in sham).

The tubular injury and apoptosis in kidney tissues were also evaluated. PAS staining showed the morphological changes in SA-AKI mice when compared with shamed operated groups,

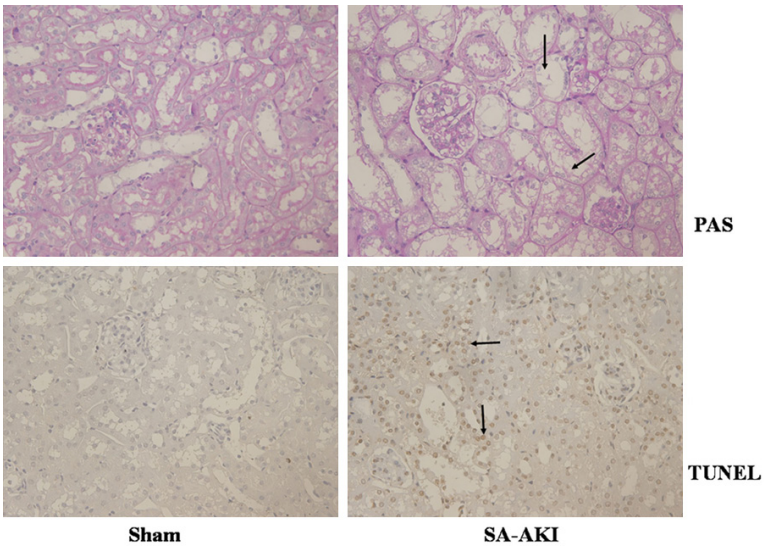

Figure 1. Histological examination of the injury in renal tissues from sham and SA-AKI mice. Top panel: PAS staining. Below panel: TUNEL staining. Kidney tissues were fixed in 10% formaldehyde for at least 24 h. 4 μm thickness specimens were subjected to PAS staining and Tunel assay for tubular injury and apoptosis assessment.

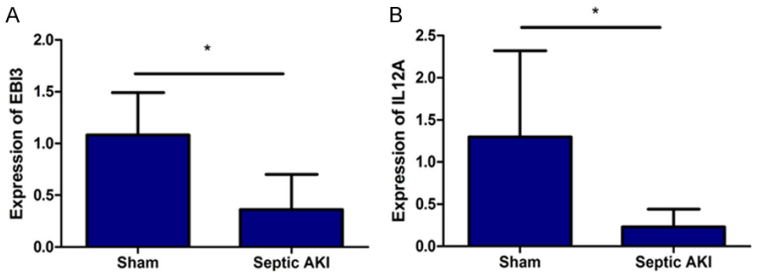

Figure 2. Real-time RT-PCR assay for mRNA expression of IL-35 subunits EBI3 (A) and IL-12α (B) in SA-AKI mice and sham groups. Each sample was conducted in triplicate. The results were analyzed using the $2^{-\Delta\Delta Ct}$ method. All samples were normalized to GAPDH as an endogenous control. *P<0.05 vs. sham compared by unpaired Student's t test.

in terms of brush border loss, tubular degeneration, and vacuolization in the proximal tubules (**Figure 1** top panel). Moreover, TUNEL assay showed more positively stained tubular epithelial cells in SA-AKI mice when compared with sham group, indicating CLP induced apoptosis in kidney tissues.

IL-35 subunits EBI3 and IL-12α mRNA expressions in renal tissues from SA-AKI mice and sham groups

IL-35 mRNA expression in renal tissues from SA-AKI mice and sham groups was evaluated by qRT-PCR. It was found that the expression levels of IL-35 subunits (EBI3 and IL-12α) mRNA were significantly down-regulated in kidney samples from SA-AKI mice compared with sham (P<0.05 for both IL-12α and EBI3) (**Figure 2A** and **2B**).

IL-35 subunits EBI3 and IL-12α protein expressions in renal tissues from SA-AKI mice and sham groups

The expression patterns of IL-35 subunits EBI3 and IL-12α in renal tissues were examined by immunohistochemistry and immunofluorescence. Both EBI3 and IL-12α were found to be expressed in the tubular epithelial cells in kidney tissues from sham and SA-AKI mice, but the expression levels of EBI3 and IL-12α were observed decreased in SA-AKI mice compared with sham groups (**Figure 3A**). Immunofluorescence result also showed that both IL-35 subunits EBI3 and IL-12α were expressed in the tubular epithelial cells in kidney from SA-AKI mice and sham controls (**Figure 3B**).

Correlation of serum Cr, BUN, ALT and AST levels with IL-35 protein levels in renal tissues in SA-AKI mice

The protein expression levels of IL-35 in renal tissues in SA-AKI mice and sham groups were further determined by ELISA (**Figure 4A**). The results showed that renal IL-35 expression levels in SA-AKI mice were significantly lower than those in sham groups (P<0.05), which was consistent with the mRNA expression pattern of IL-35. To determine the involvement and significance of IL-35 in the development and progress of SA-AKI, we initiated the correlation analysis of renal IL-35 expression with blood Cr, BUN, ALT and AST levels in SA-AKI mice. It was found that renal IL-35 levels were significantly negatively related to serum Cr levels (r = -0.584, P = 0.009) (**Figure 4B**), ALT levels (r = -0.549, P = 0.015) (**Figure 4D**) and AST levels (r = -0.475, P = 0.04) (**Figure 4E**), but not significantly with

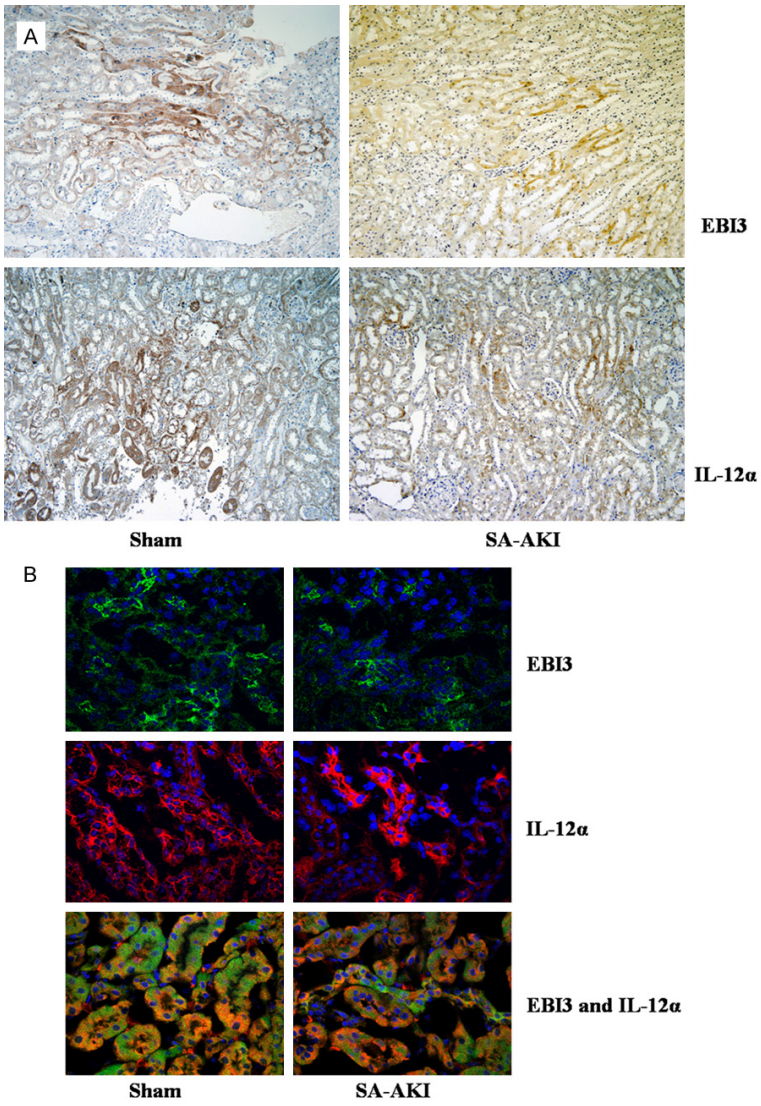

Figure 3. IL-35 subunits EBI3 and IL-12α protein expressions in renal tissues from SA-AKI mice and sham groups detected by immunohistochemistry (A) and immunofluorescence (B). (A) 4 μm thick sections from renal tissues were incubated with 1:500 anti-mouse EBI3 rat monoclonal antibody and 1:2000 anti-mouse IL-12α rabbit monoclonal antibody. The sections were counterstained with hematoxylin and mounted for immunohistochemistry observation. (B) 5 μm thick frozen sections were incubated with 1:2000 rabbit monoclonal antibody against mouse IL-12α and 1:200 rat monoclonal antibody raised against mouse EBI3, followed by secondary antibodies (1:400 dilution for Cy3-conjugated goat anti-rabbit IgG, and 1:50 dilution for FITC-conjugated donkey anti-rat IgG). Cell nuclei were stained with 0.1 μg/ml DAPI. The slides were observed under a laser scanning confocal microscope.

serum BUN levels (r = -0.437, P = 0.061) (**Figure 4C**). These results suggested that the levels of IL-35 in renal tissues are significantly related with the renal and liver damage induced by CLP.

Discussion

Sepsis and septic shock are the main causes of AKI in patients with severe sepsis. So far, no effective and safe treatment is available for AKI in clinical septic patients [1]. Common treatment methods such as fluid resuscitation, parenteral antibiotic therapy, and administration of vasopressor agents have been demonstrated not very effective in reducing the incidence of SA-AKI and its associated mortality [10]. The immune suppression has been believed to be among the key pathogenic factors in acute kidney injury, which points out the new direction for the treatment of AKI through restoring the balance and function of immune system [11, 12].

Treg cells, also known as suppressor T cells, are a subtype of T cells which modulates the immune system by suppressing the induction and proliferation of effector T cells [13]. It is reported that Tregs could ameliorate sepsis in an animal model of AKI [14], which indicates the protective effects of Tregs and the potential of Tregs-based immunotherapy in the treatment of AKI. Although the modulation of the function of Tregs is an important immunotherapeutic target, the molecules mediating their suppressive activity remain not very clear. Some studies have pointed to the fact that a novel inhibitory cytokine IL-35, mainly secreted by CD4+CD25+Tregs, confers regulatory activity on T cells [2]. So far, very little literature on the role of IL-35 in SA-AKI is available. To our knowledge, this is the first study to investigate the expression change of IL-35 in AKI animal models.

We employed cecal ligation and puncture method to generate SA-AKI model in mice. The biochemical assay confirmed the liver and renal malfunctions. The apoptosis of renal tubular epithelial cells was significantly associated with

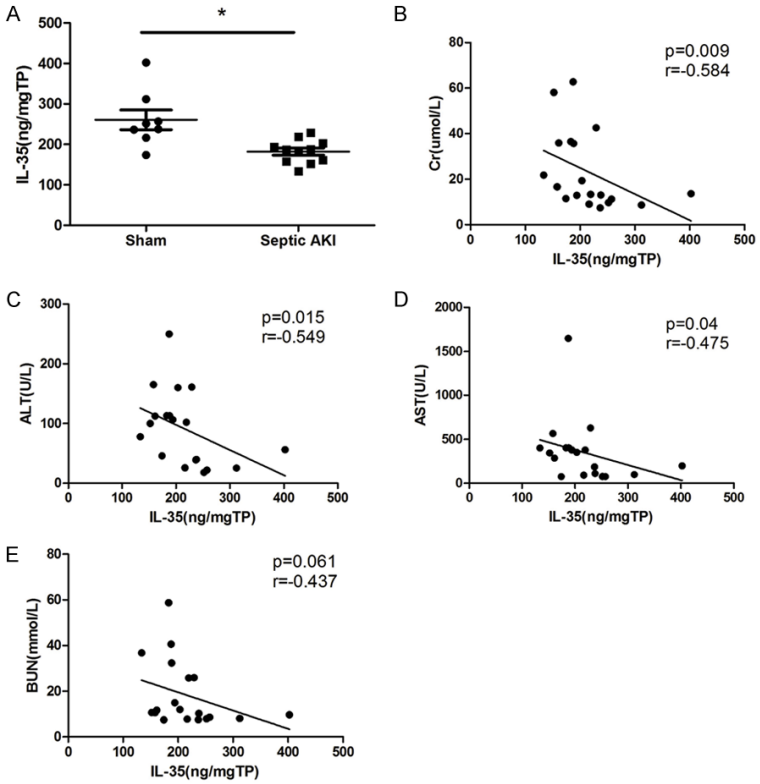

Figure 4. IL-35 protein levels in renal tissues in SA-AKI mice and sham groups detected by ELISA (A) and correlation assay of serum Cr, BUN, ALT and AST levels with IL-35 protein levels in renal tissues in SA-AKI mice (B-E). All data were expressed as mean ± SEM. (A) The differences of IL-35 levels in renal tissues between SA-AKI mice and the control groups were analyzed by unpaired Student's t test. *P<0.05 was considered statistically significant. (B-E) Correlation analysis was carried out using Pearson's method. Statistically significant negative correlation was found between renal IL-35 protein levels with serum Cr, ALT, AST levels. No statistically significant correlation was found between renal IL-35 levels with serum BUN levels.

the development AKI. This data is supported by the study from Lee et al., in which the tubular cell apoptosis was found prominent, and caspase-3 activity was positively correlated with renal dysfunction in AKI [11]. The histological examination in our study also showed injury in renal tissues in SA-AKI mice. It has been reported that IL-35 expression levels are correlated with cell apoptotic levels. Nicholl et al. found that IL-35 promoted the growth of pancreatic cancer cells and inhibited cell apoptosis [15]. Moreover, circulating IL-35 levels in non-small cell lung cancer patients were found to have an inverse correlation with overall survival [16]. Therefore, it is speculated that IL-35 may play a protective role against apoptosis of renal tubular epithelial cells in the development of SA-AKI.

It is well accepted that IL-35 is mainly secreted by Treg cells, and exerts its functions of immune suppression and anti-inflammation by inducing Treg cells and inhibiting T helper Th17 cells. Nevertheless, some studies have found that other cells, e.g. monocytes and tumor cells, can secrete IL-35 too [17]. In our study, two subunits of IL-35: EBI3 and IL-12α, were found to co-express in renal tubular epithelial cells. The decreased mRNA and protein expression of IL-35 subunits EBI3 and IL-12α was examined in renal tissues by qRT-PCR and immunohistochemistry. The protein levels of IL-35 in renal tissues of SA-AKI mice were significantly lower than those in sham controls confirmed by ELISA, which was consistent with the changes in mRNA expression levels of IL-35 detected by qRT-PCR. Moreover, the renal IL-35 levels in SA-AKI mice were found to be negatively correlated with the renal and liver functions, suggesting that the lower expression of IL-35 was associated with the development of AK-AKI. These results indicated that renal tubular epithelial cells could express IL-35, and IL-35 may be a novel target for the treatment of SA-AKI.

A lot of evidence in other diseases supported our speculation of the potential protective effects of IL-35 in AKI. Li et al. found that administration of IL-35 elevated the numbers of CD4+CD25+Foxp3+ Tregs in lungs and significantly reduced the severity of allergic airway inflammation in a murine model of asthma [18]. His observation was confirmed by a study in clinical patients with asthma, which found that the level of serum IL-35 was significantly lower when compared with the normal healthy population, and increased along with the progress in disease control [19]. Similar results were also observed in patients with dilated cardiomyopathy, who showed lower peripheral blood mononuclear cells and IL-35 levels than those in normal healthy people [20]. These results indirectly supported our findings that IL-35 expression levels were lower in AKI mice and negatively correlated with the development of AKI. The

protective effects of IL-35 have been demonstrated in clinical patients with collagen induced arthritis [21] and autoimmune diabetes [22, 23].

Nevertheless, the functions of IL-35 were found to vary in different tissues and different diseases, and some reports showed controversial results. For example, Kong et al. found that patients with active tuberculosis showed elevated serum IL-35 level and the induced proliferation of Th1 cells in order to effectively remove the pathogen [24]. Shi et al. also demonstrated that IL-35 levels in peripheral blood of patients with hepatitis B were significantly increased compared with control subjects [25]. The discrepancy in these literatures might be due to individual differences in immune status and regulation or the sample collection time in different stage of disease development.

In summary, in an animal model of SA-AKI induced by CLP, two subunits of IL-35: EBI3 and IL-12α, were co-expressed in the tubular epithelial cells in kidney tissues from both sham and AKI mice. The mRNA and protein expression levels of EBI3 and IL-12α were lower significantly in renal tissues from AKI mice when compared with sham controls. Renal total IL-35 levels were also significantly decreased in SA-AKI mice, and displayed a significantly negative correlation with the liver and renal functions. Our results indicated that IL-35 is associated with the pathological process of SA-AKI, and might represent a potential agent for SA-AKI treatment.

Acknowledgements

This research was supported by a grant from the National key research & development (R& D) plan (2016YFC1101403), Fund of Beijing Municipal Science & Technology Commission (Z131107002213011), Fund of Chinese PLA 12th Five-Year Plan for Medical Sciences (BWS14J040), and National Natural Science Foundation of China (81670694).

Disclosure of conflict of interest

None.

Address correspondence to: Xiangmei Chen, Department of Nephrology, Chinese PLA General Hospital, Chinese PLA Institute of Nephrology, State Key Laboratory of Kidney Diseases, National Clinical Research Center for Kidney Diseases, Beijing 100853, China. Tel: +86-10-55499222; Fax: +86-10-55499220; E-mail: xiangmei_chen@hainan.net; Jijun Li, First Hospital Affiliated to The Chinese PLA General Hospital, Beijing 100048, China. E-mail: lishi_han@hainan.com

References

[1] Umbro I, Gentile G, Tinti F, Muiesan P, Mitterhofer AP. Recent advances in pathophysiology and biomarkers of sepsis-induced acute kidney injury. J Infect 2016; 72: 131-42.

[2] Collison LW, Workman CJ, Kuo TT, Boyd K, Wang Y, Vignali KM, Cross R, Sehy D, Blumberg RS, Vignali DA. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. Nature 2007; 450: 566-9.

[3] Jones LL, Chaturvedi V, Uyttenhove C, Van Snick J, Vignali DA. Distinct subunit pairing criteria within the heterodimeric IL-12 cytokine family. Mol Immunol 2012; 51: 234-44.

[4] Li X, Mai J, Virtue A, Yin Y, Gong R, Sha X, Gutchigian S, Frisch A, Hodge I, Jiang X, Wang H, Yang XF. IL-35 is a novel responsive anti-inflammatory cytokine--a new system of categorizing anti-inflammatory cytokines. PLoS One 2012; 7: e33628.

[5] Niedbala W, Wei XQ, Cai B, Hueber AJ, Leung BP, McInnes IB, Liew FY. IL-35 is a novel cytokine with therapeutic effects against collagen-induced arthritis through the expansion of regulatory T cells and suppression of Th17 cells. Eur J Immunol 2007; 37: 3021-9.

[6] Ning-Wei Z. Interleukin (IL)-35 is raising our expectations. Rev Med Chil 2010; 138: 758-66.

[7] Huang CH, Loo EX, Kuo IC, Soh GH, Goh DL, Lee BW, Chua KY. Airway inflammation and IgE production induced by dust mite allergen-specific memory/effector Th2 cell line can be effectively attenuated by IL-35. J Immunol 2011; 187: 462-71.

[8] Whitehead GS, Wilson RH, Nakano K, Burch LH, Nakano H, Cook DN. IL-35 production by inducible costimulator (ICOS)-positive regulatory T cells reverses established IL-17-dependent allergic airways disease. J Allergy Clin Immunol 2012; 129: 207-15, e1-5.

[9] Wenbin Z, Guojun G. Resveratrol ameliorates diabetes-induced renal damage through regulating the expression of TGF-beta1, collagen IV and Th17/Treg-related cytokines in rats. West Indian Med J 2014; 63: 20-5.

[10] Swaminathan S, Rosner MH, Okusa MD. Emerging therapeutic targets of sepsis-associated acute kidney injury. Semin Nephrol 2015; 35: 38-54.

[11] Lee SY, Lee YS, Choi HM, Ko YS, Lee HY, Jo SK, Cho WY, Kim HK. Distinct pathophysiologic

mechanisms of septic acute kidney injury: role of immune suppression and renal tubular cell apoptosis in murine model of septic acute kidney injury. Crit Care Med 2012; 40: 2997-3006.
[12] Martina MN, Noel S, Bandapalle S, Hamad AR, Rabb H. T lymphocytes and acute kidney injury: update. Nephron Clin Pract 2014; 127: 51-5.
[13] Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 2006; 441: 235-8.
[14] Wang Y, Tao Y. Research progress on regulatory T cells in acute kidney injury. J Immunol Res 2015; 2015: 174164.
[15] Nicholl MB, Ledgewood CL, Chen X, Bai Q, Qin C, Cook KM, Herrick EJ, Diaz-Arias A, Moore BJ, Fang Y. IL-35 promotes pancreas cancer growth through enhancement of proliferation and inhibition of apoptosis: evidence for a role as an autocrine growth factor. Cytokine 2014; 70: 126-33.
[16] Gu X, Tian T, Zhang B, Liu Y, Yuan C, Shao L, Guo Y, Fan K. Elevated plasma interleukin-35 levels predict poor prognosis in patients with non-small cell lung cancer. Tumour Biol 2015; 36: 2651-6.
[17] Long J, Zhang X, Wen M, Kong Q, Lv Z, An Y, Wei XQ. IL-35 over-expression increases apoptosis sensitivity and suppresses cell growth in human cancer cells. Biochem Biophys Res Commun 2013; 430: 364-9.
[18] Li Y, Pan X, Peng X, Li S, Zhou Y, Zheng X, Li M. Adenovirus-mediated interleukin-35 gene transfer suppresses allergic airway inflammation in a murine model of asthma. Inflamm Res 2015; 64: 767-74.
[19] Yamamoto Y, Negoro T, Hoshi A, Wakagi A, Shimizu S, Banham AH, Ishii M, Akiyama H, Kiuchi Y, Sunaga S, Tobe T, Roncador G, Itabashi K, Nakano Y. Impaired Ca(2)(+) regulation of CD4(+)CD25(+) regulatory T cells from pediatric asthma. Int Arch Allergy Immunol 2011; 156: 148-58.
[20] Li J, Wang L, Wang S, Zhu H, Ye P, Xie A, Shen B, Liu C, Guo C, Fu Q, Zhang K, Xia J. The Treg/Th17 imbalance in patients with idiopathic dilated cardiomyopathy. Scand J Immunol 2010; 71: 298-303.
[21] Kochetkova I, Golden S, Holderness K, Callis G, Pascual DW. IL-35 stimulation of CD39+ regulatory T cells confers protection against collagen II-induced arthritis via the production of IL-10. J Immunol 2010; 184: 7144-53.
[22] Cai Z, Wong CK, Dong J, Chu M, Jiao D, Kam NW, Lam CW, Tam LS. Remission of systemic lupus erythematosus disease activity with regulatory cytokine interleukin (IL)-35 in Murphy Roths Large (MRL)/lpr mice. Clin Exp Immunol 2015; 181: 253-66.
[23] Mondanelli G, Volpi C, Bianchi R, Allegrucci M, Talesa VN, Grohmann U, Belladonna ML. Islet antigen-pulsed dendritic cells expressing ectopic IL-35Ig protect nonobese diabetic mice from autoimmune diabetes. Cytokine 2015; 75: 380-8.
[24] Kong B, Liu GB, Zhang JA, Fu XX, Xiang WY, Gao YC, Lu YB, Wu XJ, Qiu F, Wang WD, Yi LL, Zhong JX, Chen ZW, Xu JF. Elevated serum IL-35 and increased expression of IL-35-p35 or -EBI3 in CD4(+)CD25(+) T cells in patients with active tuberculosis. Am J Transl Res 2016; 8: 623-33.
[25] Shi M, Wei J, Dong J, Meng W, Ma J, Wang T, Wang N, Wang Y. Function of interleukin-17 and -35 in the blood of patients with hepatitis B-related liver cirrhosis. Mol Med Rep 2015; 11: 121-6.