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Original Article

Jianpi Qinghua decoction improves renal function in a rat model of adriamycin-induced chronic renal failure

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Abstract: Jianpi Qinghua decoction (JPQH), a modified formulation of a classic Chinese prescription named Sengyang decoction, was clinically used to reverse kidney fibrosis in chronic renal failure. The present study was designed to examine whether JPQH has a protective effect on the renal function associated with phosphorylated nuclear factor-κB (p-NF-κB), tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), intercellular adhesion molecular-1 (ICAM-1), interleukin-17a (IL-17a), and T helper cells 17 (Th17) in a rat model of adriamycin-induced focal segmental glomerulosclerosis (FSGS). Rat models of FSGS nephropathy were established by left nephrectomy and intravenous injection of adriamycin (ADR). Rats were then treated with JPQH. The levels of serum creatinine (Scr), blood urea nitrogen (BUN), and 24-hour urine protein quantity (24-HUPQ) were measured. The renal expressions of p-NF-κB, TNF-α, IL-6, ICAM-1, IL-17a, and Th17 were detected. JPQH significantly decreased the levels of Scr, BUN, 24-HUPQ, Th17, IL-17a mRNA, p-NF-κBp65, TNF-α, IL-6, and ICAM-1 compared to the control group ($P < 0.01$). JPQH significantly attenuated renal damage. These results suggest that JPQH can protect renal function and may be a promising agent for treating FSGS.

Keywords: Jianpi Qinghua decoctions, adriamycin-induced focal segmental glomerulosclerosis, renal fibrosis, Th17

Introduction

Focal segmental glomerulosclerosis (FSGS) is a major cause of idiopathic nephrotic syndrome and end-stage kidney disease. Elevated cytokine levels and an inappropriate balance of $CD4^+$ T-cells in peripheral blood point toward an immune-mediated injury [1-3]. Some traditional Chinese herbal formulations, including Jianpi Qinghua (JPQH) decoctions or monographs documented in the ancient Chinese Pharmacopoeia have been used in the treatment of chronic renal failure (CRF), especially in the early or middle stages of chronic kidney disease (CKD) [4-7].

JPQH is composed of radix codonopsis (Dangsen), radix astragali (Huangqi), fructus tsaoko (Caoguo), rhizoma atractylodis lanceae (Cangzhu), rhizoma coptidis (Huanglian), and radix et rhizoma rhei palmati (Dahuang). This decoction originated from Piwei Lun of Dongyuan Li. Compared to JPQH, Sengyang decoction uses radix ginseng and radix scutellariae baicalensis instead of radix codonopsis and radix et rhizoma rhei palmate, respectively. Sengyang decoction also contains radix bupleuri chinensis, radix glycyrrhizae preparata Chinensis, incised notopterygium rhizome and root, rhizoma cimicifugae foetidae but does not contain fructus tsaoko. Recent clinical studies have demonstrated that treatment with JPQH decoctions can significantly improve renal function, ameliorate proteinuria, and has anti-fibrotic property in patients with CKD [6, 8]. To further investigate the therapeutic effects and the underlying renoprotective mechanism of JPQH in reversing immune disorders, we established a rat model of FSGS by left kidney nephrectomy and tail vein injection of adriamycin (ADR).

Materials and methods

Animals

Sprague Dawley rats weighing 200 ± 20 g (BiKai, Shanghai, China), were used in the study. All the rats were individually housed in stainless

steel wire-mesh cages and fed *ad libitum* with standard solid chow (BiKai Animal Lab. Company, Shanghai, China) containing 24.5% protein. Room temperature and humidity conditions were maintained at 24 ± 1°C and 45 ± 15%, respectively. All the procedures were conducted in strict accordance with the People's Republic of China legislation on the use and care of laboratory animals, and the guidelines established by the Institute for Experimental Animals of Shanghai University of Traditional Chinese Medicine. All procedures were also approved by the University Committee for animal experiments.

Preparation of JPQH decoctions

JPQH is composed of 15 g of radix codonopsis (from the province of Shanxi), 15 g of radix astragali (from the province of Neimenggu), 6 g of fructus tsaoko (from the province of Yunnan), 10 g of rhizoma atractylodis lanceae (from the province of Neimenggu), 3 g of rhizoma coptidis (from the province of Sichuan), 9 g of and radix et rhizoma rhei palmati (from the province of Gansu). All the herbal drugs were purchased from Shanghai Huayu Pharmaceutical Co., and identified by authority. The air-dried herbs were powdered and extracted with hot water for 3 h. The extract was concentrated, precipitated four times with alcohol, and the solvent was allowed to evaporate completely. Finally, the precipitate was dissolved in hot water and cooled prior to being administered to the rats. The final yield of the prepared decoction was above 35%.

Experimental procedures

Animals were divided into three groups: sham group (normal sham-control), vehicle control group, treated group (JPQH-treated group), eight rats per each group. A left nephrectomy was performed through the translumbar route as previously described, with slight modifications [9, 10]. These procedures were performed under sodium pentobarbital anaesthesia (Shanghai Pharmaceutical Factory, Shanghai, China). One week after the left nephrectomy, the rats were treated with ADR at a dose of 3 mg/kg, and this was repeated 4 weeks later. Two weeks after the second injection of ADR, rats with an increase in the serum creatinine (Scr) levels were treated with a daily dose of JPQH (3.9 g/200 g body weight) or vehicle for 8 weeks.

Assessment of renal function

Scr and blood urea nitrogen (BUN) levels were measured using a Beckman Cx4 analyser (Fullerton, CA, USA). Twenty-four-hour urinary protein (24-HUP) concentrations were determined by the Bradford method, adapted to a microtiter plate assay. Coomassie reagent (USB, Cleveland, OH) was added to the diluted urine samples. After 10 min, the absorbance was read at 595 nm on an ELX800 microplate reader (Bio-Tek Instruments, VT). The protein concentrations were calculated by reference to bovine serum albumin (Sigma) standards.

Assessment using a transmission electron microscope (TEM)

Transmission electron microscope (TEM) is extensively used in medical applications to reveal the ultrastructure of biological samples. Images were acquired using a Gatan Model 782 system with magnification from 0 to 6000. Images were then digitally captured by a TECNAI 12 Bio TWIN (Philips) charge-coupled device camera with 8 bits per pixel.

Western blots analysis

Kidney tissue samples were lysed in lysate containing 1 μg/mL leupeptin, 1 μg/mL aprotinin, and 100 μmol/L phenylmethylsulphonyl fluoride (PMSF). The lysates were clarified by centrifugation at 12,000 rpm for 30 min at 4°C, and the protein concentration in each lysate was determined using a BCA protein assay kit (Pierce, Rockford, IL, USA). Protein samples (50 mg) were separated by 6%-10% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and electroblotted onto nitrocellulose membranes. The membranes were blocked with 5% skim milk and incubated with primary antibodies for p-NF-κBp65 (Cell Signaling Technology Inc., USA), TNF-α (Abcam Inc., USA), IL-6 (Abcam Inc.), ICAM-1 (Abcam Inc.) at 4°C overnight. The membranes were then incubated with a horseradish peroxidase-conjugated secondary antibody. Immunoreactive bands were visualised by staining with ECL reagent and exposed to X-ray film.

Real-time quantitative polymerase chain reaction (qPCR) analysis for IL-17a

To investigate the mRNA expression of IL-17a, one-step qPCR (BC Living Creature Technique

Table 1. Effect of JPQH on serum creatinine (Scr) (umol/L)

Group	*n*	Scr	Before treatment	After treatment
Sham	8	*M*	43.80	45.97
		min	31.07	14.52
		max	62.42	66.14
Control	7	*M*	185.83	343.95
		min	100.61	169.35
		max	228.84	524.60
JPQH	7	*M*	174.39	180.65
		min	118.36	77.82
		max	253.30	346.37

Co., Shanghai, China) was performed using the Opticon machine (FX Scientific Research Inc., Shanghai, China). Briefly, total RNA was extracted from renal tissues by homogenisation in Beads (Beads RNA extraction kit, TOYOBO Inc., Shanghai, China). All the RNA samples were treated with RNase-free DNase I (GIBCO BRC Inc., Shanghai, China) prior to RT-PCR analysis. Real-time quantitative one-step RT-PCR assay was performed to quantify mRNA using real-time PCR machine (FX Scientific Research Inc., Shanghai, China). The primers used for real-time qPCR were as follows: IL-17a, forward 5-CTGATCA GGA CGA GCG ACC A-3; reverse 3-ACGATGACTTGGACCTCCGATGTC A-5. Quantitative analyses of IL-17a expressions were performed using a quantitative image analysis system FR-2000 (FR Science and Technology Inc., Shanghai, China).

Th17 analysis by flow cytometry (FCM)

Intracellular staining was performed with the BD Cytofix/cytoperm kit, according to the manufacturer's instructions. Samples were acquired in a FACSCalibur flow cytometer (Becton Dickinson Company, USA) and the analysis was performed with Flowjo version 7.2.2 (Treestar, Inc.). The analysis of cell populations and cytokine production was conducted by using a first gate that included live lymphocytes as determined by forward scatter (FSC) and side scatter (SSC) characteristics. Next, T-cells (gated according to CD3 expression) were plotted in a CD4 vs. IL-17a graphic that allowed the identification of discrete CD4 and IL-17a populations. Subsequent analyses were performed on these cell populations. In some experiments, cells were stained with anti-CD4-APC and anti-IL-17a PE-Cy7 and sorted in a FACSAria flow cytometer (Becton Dickinson Company).

Statistical analysis

Statistical analyses were performed using SPSS 18.0 software and GraphPad Prism 3.0 (GraphPad Software, Inc., San Diego, CA) with $P \leq 0.05$ considered as statistically significant. Data were represented as mean ± standard deviation (SD) and conformed to a normal distribution pattern and homogeneity of variance. Groups were compared by using a single factor analysis of variance (ANOVA) and S-N-K's multiple comparisons. For data that did not conform to a normal distribution, data representation was in terms of median, minimum, and maximum. Groups were compared by using the Wilcoxon signed rank test and Nemenyi test, before and after treatment, by using generalised estimating equations.

Results

One rat from each pair was assigned to the treatment group (n = 8) and administered JPQH at a daily dose of 3.9 g/200 g (body weight) for 8 weeks. This dose was determined by the human clinical dosage. The remaining rats were allocated to the control group (vehicle control, n = 8) and received vehicle only. The sham group rats (n = 8) underwent a sham operation consisting of laparotomy and manipulation of the renal pedicles without damaging the kidney.

Prior to treatment, levels of Scr and BUN were determined. The levels of Scr, BUN, and 24-HUPQ were also determined 8 weeks after the administration of JPQH. At week 8, the animals were sacrificed, and the remnants of the kidneys were collected for histopathological and gene expression studies. The spleens were collected for flow cytometry analysis. During weeks 6 and 7 of the experiments, two rats from the control group died and a rat from the JPQH group died at week 7. At the end of the experiments, the number of animals per group was as follows: sham group, n = 8; control group, n = 6; and treatment group, n = 7.

Effects of JPQH on physiological biochemical parameters in FSGS

Renal function and 24-HUPQ levels were evaluated before and at week 8 following the administration of JPQH (**Tables 1-6**). In the control group, body weights were different before and after treatment. Average food intake was similar among the groups. At the end of the experi-

Table 2. Results of Scr analysis in three groups using Wilcoxon signed rank test and Nemenyi test

Parameter	*B*	SE	95% *Wald feasible region*		Hypothesis test		
			Minimum	Maximum	*Wald* X^2	*df*	*Sig.*
(section)	22.452	9.7174	3.407	41.498	5.339	1	0.021
[ZB = 3]	137.905	14.2309	110.013	165.797	93.907	1	< 0.01
[ZB = 2]	202.358	16.9737	169.090	235.626	142.131	1	< 0.01
[ZB = 1]	0[a]	--	--	--	--	--	--
After treatment	-9.088	48.1146	-103.391	85.215	0.036	1	0.850
Before treatment	-42.330	46.8888	-134.230	49.571	0.815	1	0.367
(graduation)	4665.740	--	--	--	--	--	--

ZB = 1, Sham; ZB = 2, Control; ZB = 3, JPQH. a: 0.

Table 3. Effect of JPQH on serum blood urea nitrogen (BUN) μmol/L

Group	*n*	BUN	Treatment	
			Before	After
Sham	8	*M*	5.27	5.12
		min	4.39	4.65
		max	7.34	6.19
Control	7	*M*	13.89	21.38
		min	10.79	12.18
		max	18.86	22.69
JPQH	7	*M*	14.28	13.54
		min	10.30	5.59
		max	19.80	18.44

ment, there was a significant difference in the body weight between the control FSGS and the JPQH-treated FSGS rats, although they were pair-fed. The body weight of JPQH-treated rats was significantly higher than the vehicle-treated ones. The progression of renal function deterioration showed the highest expression of Scr and BUN at the end of the experimental period, while these parameters were markedly restored in JPQH-treated rats at week 8. Urinary protein excretion in JPQH-treated rats was also suppressed obviously at week 8 as compared with FSGS rats in the control group.

Effects of JPQH on protein expressions of p-NF-κB, TNF-α, IL-6, and ICAM-1 in renal tissue

Western blot analysis was used to quantify the expressions of p-NF-κB, TNF-α, IL-6, and ICAM-1 protein in the kidney tissue remnants (**Table 7**; **Figures 1**-**4**). In the control group, there was a substantial increase in the renal expression of p-NF-κB following ADR administration, which was attenuated by treatment with JPQH (P < 0.01, **Table 7**; **Figure 2**). Furthermore, the protein levels of TNF-α, IL-6, and ICAM-1 in the renal cortex of the JPQH-treated group were also attenuated as compared to the control group (P < 0.01, **Table 7**; **Figures 2**-**4**). The variation in protein levels of p-NF-κB, TNF-α, IL-6, and ICAM-1 among the groups might be attributable to the variation in the progression of CKD.

Effects of JPQH on the frequency of Th17 cells in renal tissue

Th17 cells and cytokines participate in human and experimental renal disease, especially in proliferative glomerulonephritis where Th17 effector cells are active [11]. Th17 cells can also induce direct glomerular injury. Our results showed that the frequency of Th17 cells was significantly higher (P < 0.01) in the control group as shown in **Table 8** and **Figure 5**. The frequency of Th17 cells was significantly decreased following treatment with JPQH decoctions (P < 0.01, **Table 8**; **Figure 5**). Collectively, these results suggest that JPQH downregulated the expression of Th17 cells, and this may be relevant to the persistent immune-mediated injury.

Effects of JPQH on IL-17a mRNA levels in renal tissue

JPQH treatment significantly reversed the ADR-mediated upregulation of renal mRNA levels of IL-17a (P < 0.01, **Table 8** and **Figure 6**).

Discussion

FSGS occurs in combination with extracellular matrix deposition in the tubulointerstitium. It is a well-defined clinicopathological entity that has multiple causes and pathogenic mechanisms. Immune mechanisms have been postu-

Table 4. Results of BUN analysis in three groups by using Wilcoxon signed rank test and Nemenyi test

Parameter	*B*	SE	95% *Wald feasible region*		Hypothesis test		
			Minimum	Maximum	*Wald* X^2	*df*	*Sig.*
(section)	4.841	0.3952	4.066	5.615	150.013	1	< 0.01
[ZB = 3]	8.415	0.9128	6.626	10.205	84.993	1	< 0.01
[ZB = 2]	10.875	0.8812	9.148	12.603	152.329	1	< 0.01
[ZB = 1]	0[a]	--	--	--	--	--	--
After treatment	1.317	0.8735	-0.395	3.029	2.274	1	0.132
Before treatment	0[a]	--	--	--	--	--	--
(graduation)	9.292	--	--	--	--	--	--

ZB = 1, Sham; ZB = 2, Control; ZB = 3, JPQH. a: 0.

Table 5. Effects of JPQH on 24 h urinary protein excretion (24-HUPQ) mg/24 h

Group	*n*	24-HUP	Treatment	
			Before	After
Sham	8	*M*	8.51	6.26
		min	3.96	4.31
		max	12.10	14.50
Control	7	*M*	42.58	59.63
		min	35.24	36.38
		max	64.60	98.67
JPQH	7	*M*	41.79	47.77
		min	32.47	44.29
		max	56.65	52.83

lated in the pathogenesis of FSGS. Several profibrogenic cytokines such as TNF-α, IL-6, IL-17a, and ICAM-1 play key roles in the development of renal fibrosis through the Th17/p-NF-κB signalling pathway.

NF-kB, a family of dimeric transcription factors that regulate the expression of numerous genes involved in inflammation and cell proliferation, has been found activated in experimental renal diseases. It is now widely acknowledged that the anti-inflammatory action of steroids is basically mediated through inhibition of the transactivation of NF-kB-dependent genes. NF-kB therefore, plays a central role in inflammatory diseases, including kidney diseases [12].

Recent studies have demonstrated that Th17 cell subsets can secrete cytokines such as IL-17a, IL-6 and TNF-α. Unlike those produced by Th1 and Th2 cell subsets, cytokines produced by Th17 subsets may function in the collection, mobilisation, or promotion of neutrophil recruitment [13, 14]. Th17 cells, a subset of $CD4^+$ effector lymphocytes, play a role in cell-mediated autoimmune diseases and some inflammatory diseases [15, 16]. Th17 cells and their effector cytokines participate in human and experimental renal disease, especially proliferative glomerulonephritis where Th17 effector cells are active [17]. Th17 cells can also induce glomerular injury directly. Th17 cells also produce IL-17a, a cytokine that serves as an immunomodulator and participates in inflammatory immune responses, immune rejection, and other biological actions [18].

It is well established that in both mice and humans with systemic lupus erythematosus (SLE), Th17 cells are hyperactivated and produce increased amounts of IL-17, which are inflammatory cytokines associated with Th1 and Th17 cells. The abundance of IL-17 was correlated with disease activity [19]. Inhibiting IL-17 production improves murine lupus nephritis. Crispin et al. reported that IL-17-producing T-cells are found in kidney biopsies from patients with lupus nephritis [20]. In C56BL/6J (B6), B6.MRL/lpr, MRL/lpr/2J (MRL), MRL/MpJ (MPJ), and Rag-1 knockout mice, Zhang found that Th17 and IL-17 were significantly increased in renal tissue [21].

Jianpi Qinghua decoctions are composed of *radix codonopsis* (Dangsen), *radix astragali* (Huangqi), *fructus tsaoko* (Caoguo), *rhizoma atractylodis lanceae* (Cangzhu), *rhizoma coptidis* (Huanglian), and *radix et rhizoma rhei palmati* (Dahuang). Its effect in eliminating heat and purging fire has been proven clinically. The various components of the decoction have different active ingredients. For *radix codonopsis,* the active ingredient is Lobetyolin [22, 23], an anti-inflammatory, anti-ulcerative polyacetylene compound [24]. The main active ingredient

Table 6. Results of 24-HUPQ analysis in three groups by using Wilcoxon signed rank test and Nemenyi test

Parameter	*B*	SE	95% *Wald feasible region*		Hypothesis test		
			Minimum	Maximum	*Wald* X^2	*df*	*Sig.*
(section)	5.250	1.3395	2.625	7.875	15.362	1	< 0.01
[ZB = 3]	36.780	1.2793	34.272	39.287	826.568	1	< 0.01
[ZB = 2]	42.700	3.3388	36.156	49.244	163.563	1	< 0.01
[ZB = 1]	0[a]	--	--	--	--	--	--
After treatment	6.937	3.0735	0.914	12.961	5.095	1	0.024
Before treatment	0[a]	--	--	--	--	--	--
(graduation)	92.484	--	--	--	--	--	--

ZB = 1, Sham; ZB = 2, Control; ZB = 3, JPQH. a: 0.

Table 7. Relative abundance of p-NF-κB, TNF-α, IL-6, and ICAM-1 in renal tissue

Group	*n*	P-NFκBp65	TNF-α	IL-6	ICAM-1
Sham	3	0.124 ± 0.032	0.282 ± 0.058	0.385 ± 0.037	0.300 ± 0.043
Control	3	1.977 ± 0.323*	1.439 ± 0.354*	1.604 ± 0.465*	1.090 ± 0.342*
JPQH	3	0.815 ± 0.168▽	0.378 ± 0.029▽	0.299 ± 0.100▽	0.207 ± 0.034▽
F	--	39.443	39.443	19.057	14.049
P	--	0.007	0.007	0.020	0.030

*$P < 0.01$ vs. sham group; ▽$P < 0.01$ vs. control group.

of *radix astragali* is astragaloside IV (AS-IV), which consists of active *Astragalus* saponin [25]. AS-IV exhibits biological activity, including immunomodulation, anti-oxidation, anti-viral, and anti-cancer effects. It also prevents cardiovascular disease and lowers blood glucose [26-29]. AS-IV can protect mesangial cells against H2O2-induced oxidative stress injury by inhibiting the p38/MAPK signalling pathway, upregulating the expression of cyclin D1, and reducing the intracellular reactive oxygen species (ROS)-mediated oxidative stress injury [27]. In a rat model of ischemia/reperfusion (IR) induced acute kidney injury (AKI), AS-IV could downregulate the mRNA expressions of NF-κB, TNF-α, MCP-1, and ICAM-1 in a rat model of acute kidney injury. These data suggest that AS-IV could be developed as a novel therapeutic approach in preventing ischemic AKI, through inhibition of NF-κB-mediated inflammatory gene expression [30]. In vivo studies have revealed that AS-IV has been proven to reduce the expression of α-SMA. The main active ingredient in *Fructus tsaoko* is the 1,8-cineole of volatile oil, which mainly prevents bacterial infection, oxidative injury, tumour development as well as regulates gastrointestinal function [31]. More than twenty polyethylene acetylenic compounds have been currently isolated from *Rhizoma atractylodis lanceae*, and atractylodin was found to be the main active compound. These components also have diverse biological properties, including anti-inflammatory and gastrointestinal protective properties [32]. The main active ingredient of *Rhizoma coptidis* is berberine, whose main functions include anti-infection, anti-cancer, lowering of blood lipids, lowering of blood glucose, and improving insulin sensitivity [33-36]. In addition, berberine can improve microcirculation, inhibit oxidative injury, and function as an immunomodulator [36]. The pharmacological effects of berberine on lipid metabolism are dependent on the activation the extra cellular signal-regulated kinases (ERK) signalling pathway via regulation of low-density lipoprotein (LDL) receptors [35]. On the other hand, berberine can inhibit the NF-kB pathway and decrease the expression of inflammatory mediators such as TNF-α, ICAM-1, TGF β-1, and iNOS [37]. In a model of diabetic kidney disease (DKD), berberine can increase superoxide dismutase (SOD) and decrease MDA levels and partially reverse renal tubulointerstitial lesions [38]. Radix et rhizoma rhei palmati, whose main active ingredient is emodin has been proven to have potential immunosuppressive, anti-inflammatory, anti-atherosclerotic, and anti-cancer activities [39]. In vit*ro* studies have revealed that emodin can inhibit renal tubular cell proliferation in a time-dependent manner. The extracts of *radix et rhizoma rhei palmati* can decrease oxidative stress and attenuate renal microinflammation

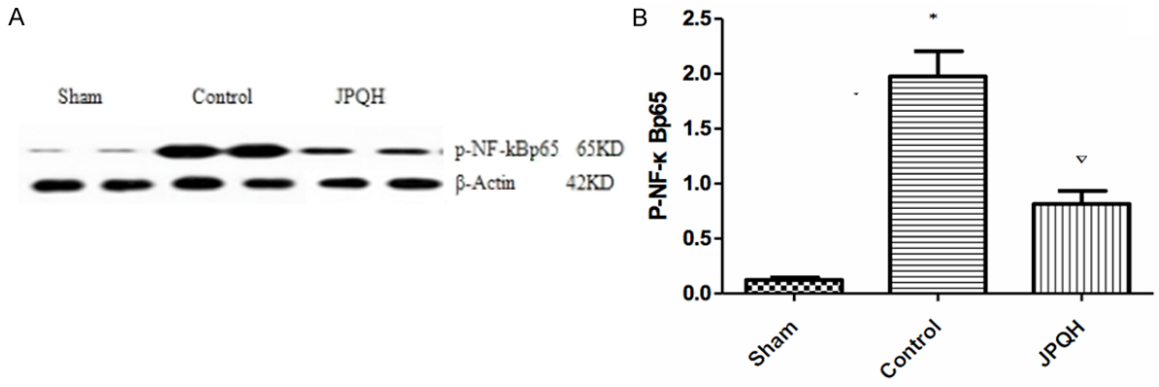

Figure 1. JPQH treatment significantly reversed the upregulation of p-NF-κB induced by adriamycin. A. Abundance of p-NF-κB in kidney tissues. The density of TNF-α band was normalised to β actin. B. Quantitation of the Western blot results. $^{*}P < 0.01$ vs. sham group, $^{\triangledown}P < 0.01$ vs. control group.

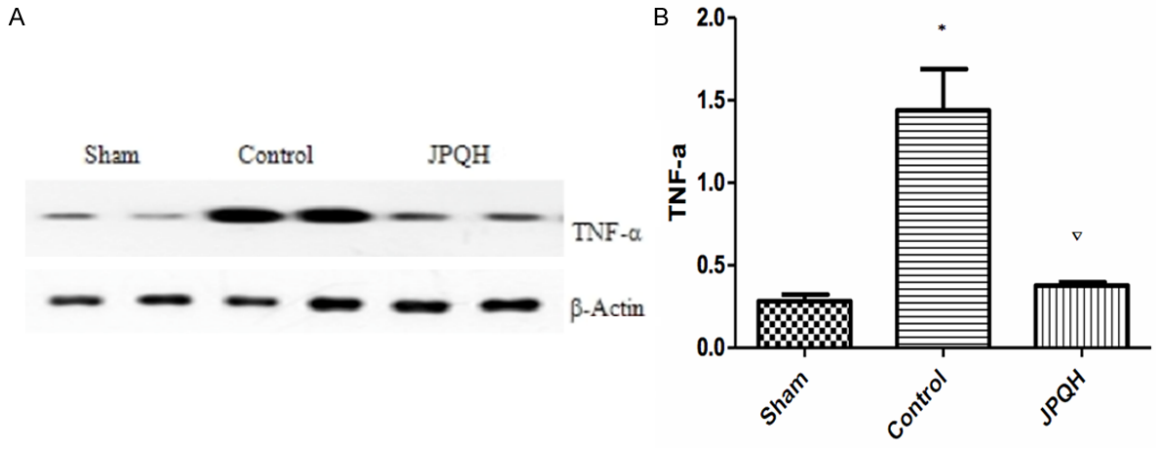

Figure 2. JPQH treatment significantly reversed the upregulation of TNF-α induced by adriamycin in renal tissue. A. Abundance of TNF-α in renal cortex. The density of TNF-α band was normalised to β actin. B. Quantitation of the Western blot results. $^{*}P < 0.01$ vs. sham group, $^{\triangledown}P < 0.01$ vs. control group.

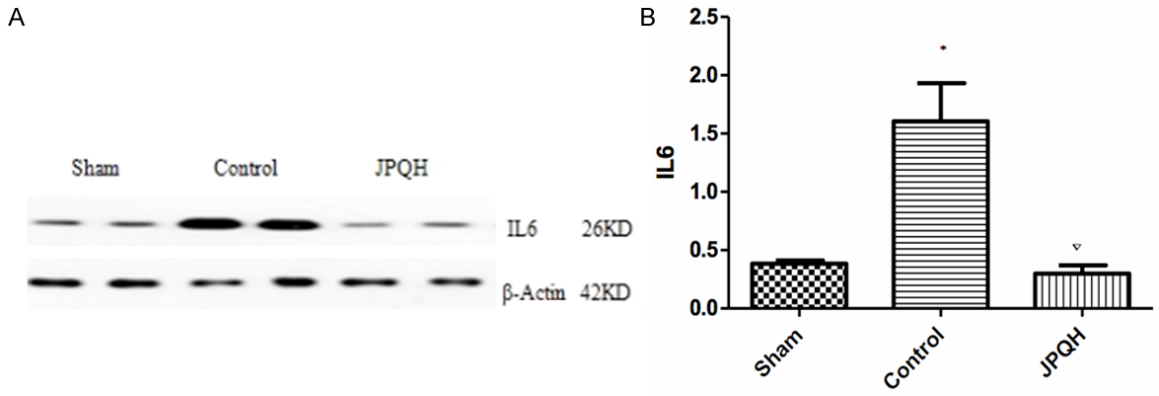

Figure 3. JPQH treatment significantly reversed the up-regulation of IL-6 induced by adriamycin in renal tissue. A. Abundance of Il-6 protein in kidney tissues. The density of TNF-α band was normalised to β actin. B. Quantitation of the western blot results. $^{*}P < 0.01$, vs. sham group, $^{\triangledown}P < 0.01$, vs. control group.

in a rat model of chronic renal failure. Studies have reported that emodin can improve renal pathological changes, delay renal fibrosis via inhibiting the phosphorylation of p38 MAPK

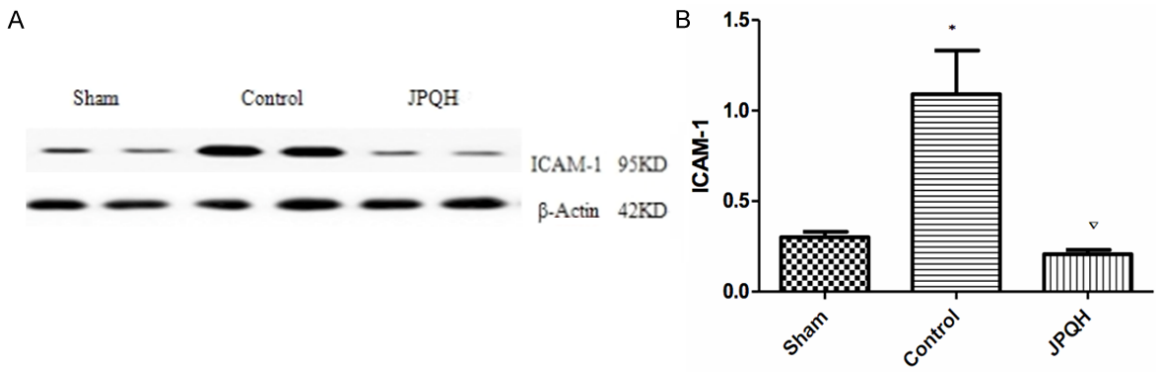

Figure 4. JPQH treatment significantly reverse the up-regulation of ICAM-1 induced by adriamycin in renal tissue. A. Abundance of ICAM-1 protein in kidney tissues. B. The density of TNF-α band was normalised to β actin. *P < 0.01, vs. sham group, ▽P < 0.01, vs. control group.

Table 8. Effects of JPQH on the mRNA levels of IL-17a and frequency of Th17 cells in renal tissue

Group	*n*	IL-17a mRNA (×10^{-5})	Th17
Sham	3	0.090 ± 0.156	0.32 ± 0.20
Control	3	4.203 ± 0.031*	3.52 ± 0.04*
JPQH	3	0.452 ± 0.009▽	1.39 ± 0.11▽
F	–	1844.341	439.487
P	–	< 0.01	< 0.01

*P < 0.01, vs. sham group, ▽P < 0.01 vs. control group.

(p-p38 MAPK) [40]. These herbs contribute to the effects of Jianpiyiqi and Qingrehuashi that might attenuate immunodepression and improve microcirculation. They also exhibit antioxidant and anti-inflammatory properties after being concocted.

JPQH decoctions were used to treat patients with chronic renal failure. It has been reported to improve cellular immune and renal functions and reduce proteinuria [41]. There is little experimental data regarding the probable molecular mechanisms of the renoprotective effects of JPQH in chronic renal failure although it is widely used in the clinical setting. Therefore, in the present study, we examined the renoprotective effects of JPQH against left kidney nephrectomy and ADR treatment. In addition, we investigated the influence of JPQH on gene expressions of Th17, p-NF-κB, IL-17a, TNF-α, IL-6 and ICAM-1.

Our results showed that the percentage of Th17 cells in the peripheral blood of the JPQH-treated group was significantly lower than that in the control group. Since Th17 cells can secrete inflammatory cytokines such as IL-17a, IL-6, and TNF-α [13, 14], treatment with JPQH may reduce the production of these cytokines by inhibiting Th17 expansion. The most notable role of IL-17 is its involvement in inducing and mediating proinflammatory responses. Based on our findings, both serum levels of IL-6 and renal IL-17a mRNA levels were significantly decreased after treatment with JPQH decoctions. This suggests that JPQH treatment might result in the suppression of cell-mediated autoimmunity and inflammatory disease by inhibiting Th17 differentiation. IL-6 together with TGF-β, substantially promotes Th17 cell development. Therefore, JPQH decoctions might slow the progression of kidney disease in the rat model of FSGS by inhibiting Th17 cell differentiation, as well as restraining the secretion of IL-6 and IL-17a. IL-17 is an initiator that the inflammatory response is T cell-induced, it can amplify the inflammatory reaction, its main biological effect is promote the inflammatory reaction [42].

In addition, protein levels of ICAM-1 and phosphorylated NF-κBp65 in the renal cortex of the JPQH-treated group were significantly decreased. IL-17 is able to activate NF-kB. After IL-17 binds to its receptors, the endothelium, epithelium, and fibroblasts are activated and overproduce IL-6 and ICAM-1 [43]. In our study, phosphorylation of NF-κBp65 as well as the activation of the NF-kB pathway was blocked after treatment with JPQH, which might be responsible for the down-regulation of Th17 cells and reduced production of IL-17a by Th17. In addition, inhibiting the NF-kB pathway fur-

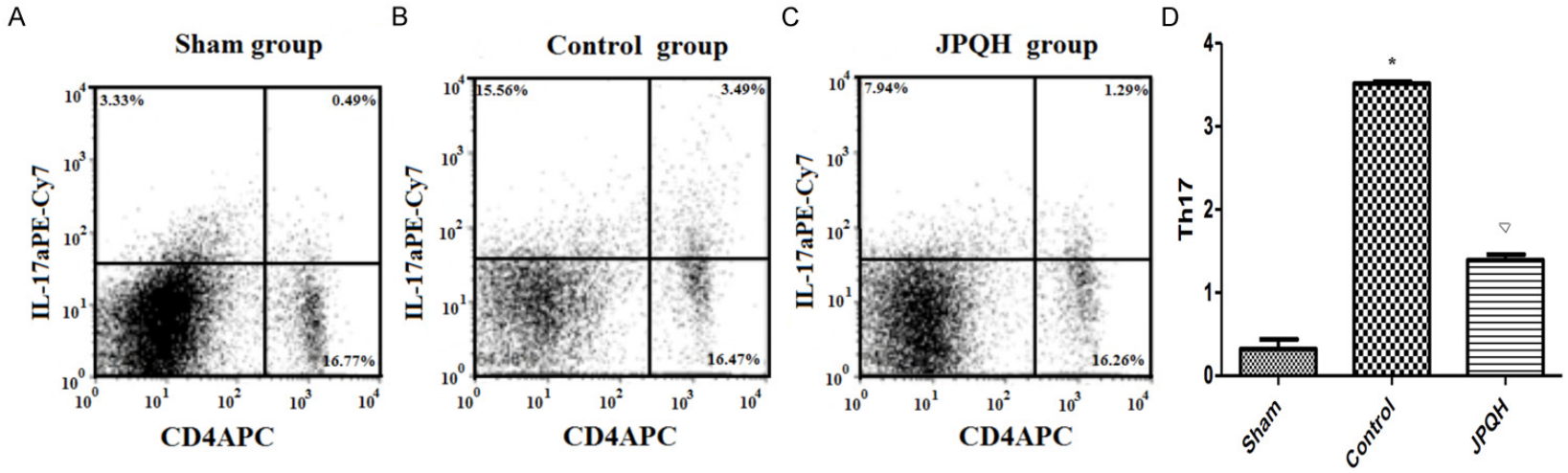

Figure 5. The frequency of Th17 cells in the rat model of FSGS. A. Representative double-stain flow profiles showing the percentage of Th17 cells ($CD4^+$ $IL\text{-}17a^+$ T cells) in the renal cortex from one sham rat. B. Representative double-stain flow profiles showing the percentage of Th17 cells in the renal cortex from a rat model of FSGS. C. Representative double-stain flow profiles showing the percentage of Th17 cells after JPQH treatment. D. Quantitation of the flow cytometric analysis. Values are mean ± SEM. $^*P < 0.01$ vs. the sham group, $^\triangledown P < 0.01$ vs. the control group.

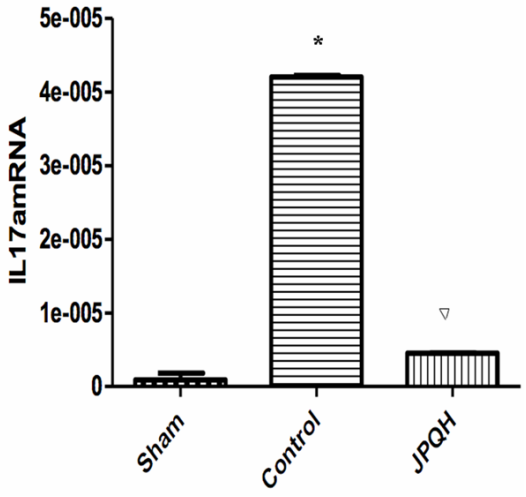

Figure 6. Relative abundance of renal IL-17a mRNA. mRNA abundance of IL-17a was significantly increased after ADR administration. JPQH treatment substantially reversed the upregulation of IL-17a. $^{*}P < 0.01$ vs. sham group; $^{\triangledown}P < 0.01$ vs. control group.

ther decreased the production of IL-6 and ICAM-1.

TNF-α, one of the main downstream effectors of NF-κB, is an adipokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. A number of studies have shown that TNF-α plays an important role in the animal models of ADR-induced nephropathy [44]. In our study, we found that the protein level of TNF-α in the remnants of the kidneys was lower in JPQH-treated rats as compared with the control group. This result is consistent with the ADR-induced FSGS model in which the NF-κB pathway plays a key role. Since TNF-α is an important mediator in the early phase of inflammation, treatment with JPQH decoction may suppress inflammation of the remaining kidney from the onset. Electron microscopy revealed that the pathological lesions in the JPQH-treated group were significantly attenuated as compared with the control group, and this was accompanied by a decrease in Scr and BUN levels.

In terms of differentiation and regulation, Th17 cells are closely involved in the downregulation of T-cells, which induces significant immunosuppressive effects. There is an inverse relationship between the pro-inflammatory IL-17a producing Th17 cells and the protective regulatory T-cells [45]. Functionally, Th17 cells and their cytokines can mediate inflammatory reactions. Th17 plays an important role in microbial infections, tissue inflammation, autoimmune diseases, and graft-versus-host diseases. JPQH decoction not only improves renal function and attenuates proteinuria in the clinical setting but also inhibits tissue inflammation at the cellular level. JPQH decoction inhibits Th17 expansion and inactivates the NF-kB pathway, thereby restraining the secretion of inflammatory cytokines such as IL-6, IL-17a, and ICAM-1. Thus, inflammation of the kidney was further suppressed and its condition subsequently improved.

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Disclosure of conflict of interest

None.

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References

[1] Summers SA, Steinmetz OM, Li M, Kausman JY, Semple T, Edgtton KL, Borza DB, Braley H, Holdsworth SR, Kitching AR. Th1 and Th17 cells induce proliferative glomerulonephritis. J Am Soc Nephrol 2009; 20: 2518-2524.

[2] Paust HJ, Turner JE, Steinmetz OM, Peters A, Heymann F, Hölscher C, Wolf G, Kurts C, Mittrücker HW, Stahl RA, Panzer U. The IL23/Th17 axis contributes to renal injury in experimental glomerulonephritis. J Am Soc Nephrol 2009; 20: 969-979.

[3] Couser WG. Basic and Translational Concepts of Immune-Mediated Glomerular Diseases. J Am Soc Nephro 2012; 23: 381-399.

[4] He LQ, Wang Y, Cao HX, Li J. The effect of kang qian ling decoction on PDGF-mRNA, TNF-mRNA expression of CRF rat renal tissue. Chinese Journal of Experimental Traditional Medical Formulae 2003; 9: 29-32.
[5] Li J, He LQ, Li Y, Hou WG. The effect of kang qian ling 2 decoction on serum lipidmetabolism of chronic renal fail rats. Journal of International Medical Science of China 2003; 3: 204-206.
[6] He LQ, Cai G. The clinical observation of the JIAN-PI-QIN-HUA prescription on spleen deficiency anddampness heat style patientswith chronic renal failure. Journal of International Medical Science of China 2005a; 3: 270-273.
[7] He LQ, Li J, Li Y. The effect of "FUZHENGHUOXUE decoction" on the expressions of fibronectin and transforming growth factor-mRNA in renal tissue of the CRF rats. Journal of Chinese Medicine 2005b; 46: 454-457.
[8] Fu Q, He LQ, Cao HX. Effects of Jianpi Qinghua Recipe on oxygen radicals and transforming growth factor β1 in renal tissue in a rat model of chronic renal failure with hyperlipidemia. Journal of Chinese Integrative Medicine 2006; 4: 408-412.
[9] Wan YG, Sun W, Dou CH. Effect of multi-glycoside of tripterygium wilfordii hook.f. in intervening TGF-β1/smad signaling pathway of adriamycin-induced nephropathy model rat. Zhongguo Zhong Xi Yi Jie He Za Zhi 2011; 31: 517-524.
[10] Ma XH, He LQ. Establishment and comparison of the focal segmental glomurular sclerosis nephrosis rat model with different dosage adriamycin to induce. Chinese Journal of Comparative Medicine 2013; 23: 37-42.
[11] Kitching AR, Holdsworth SR. The emergence of Th17 cells as effectors of renal injury. J Am Soc Nephro 2011; 22: 235-238.
[12] Muller DN, Dechend R, Mervaala EM, Park JK, Schmidt F, Fiebeler A, Theuer J, Breu V, Ganten D, Haller H, Luft FC. NF-kB inhibition ameliorates angiotensin II-induced inflammatory damage in rats. Hypertension 2000; 35: 193-201.
[13] Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, Weaver CT. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 2005; 6: 1123-1132.
[14] Cheung PF, Wong CK, Lam CW. Molecular mechanisms of cytokine and chemokine release from eosinophils activated by IL-17A, IL-17F, and IL-23: implication for Th17 lymphocytes-mediated allergic inflammation. J Immunol 2008; 180: 5625-5635.
[15] Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. Annu Rev Immunol 2009; 27: 485-517.
[16] Dong C. TH17 cells in development: an updated view of their molecular identity and genetic programming. Nat Rev Immunol 2008; 8: 337-348.
[17] Kitching AR, Holdsworth SR. The emergence of TH17 cells as effectors of renal injury. J Am Soc Nephrol 2011; 22: 235-238.
[18] Couser WG. Basic and translational concepts of immune-mediated glomerular diseases. J Am Soc Nephrol 2012; 23: 381-399.
[19] Apostolidis SA, Crispín JC, Tsokos GC. IL-17-producing T cells in lupus nephritis. Lupus 2011; 20: 120-124.
[20] Crispín JC, Oukka M, Bayliss G, Cohen RA, Van Beek CA, Stillman IE, Kyttaris VC, Juang YT, Tsokos GC. Expanded double negative T cells in patients with systemic lupus erythematosus produce IL-17 and infiltrate the kidneys. J Immunol 2008; 181: 8761-8766.
[21] Zhang Z, Kyttaris VC, Tsokos GC. The role of IL-23/IL-17 axis in lupus nephritis. J Immunol 2009; 183: 3160-3169.
[22] Han G, He X, Yang J, Yuda M, Kasai R, Otani K, Tanaka O. Chemical constituents of Codonopsis pilosula Nannf. Zhongguo Zhong Yao Za Zhi 1990; 15: 127.
[23] He Q, Zhu EY, Wang ZT, Xu LS, Hu ZB. Flavones Isolated from Codonopsis xundianensis. J Chin Pharm Sci 2004; 13: 212-213.
[24] Wang ZT, Du Q, Xu GJ, Wang RJ, Fu DZ, Ng TB. Investigations on the protective action of Condonopsis pilosula (Dangshen) extract on experimentally-induced gastric ulcer in rats. Gen Pharmacol 1997; 28: 469-473.
[25] Wang YP, Li XY, Song CQ, Hu ZB. Effect of astragaloside IV on T, B lymphocyte proliferation and peritoneal macrophage function in mice. Acta Pharmacol Sin 2002; 23: 263-266.
[26] Yesilada E, Bedir E, Caliş I, Takaishi Y, Ohmoto Y. Effects of triterpene saponins from Astragalus species on in vitro cytokine release. J Ethnopharmacol 2005; 96: 71-77.
[27] ZhangY, Zhu H, Huang C, Cui X, Gao Y, Huang Y, Gong W, Zhao Y, Guo S. Astragaloside IV exerts antiviral effects against coxsackievirus B3 by upregulating interferon-gamma. J Cardiovasc Pharmacol 2006; 47: 190-195.
[28] Li X, He D, Zhang L, Cheng X, Sheng B, Luo Y. A novel antioxidant agent, astragalosides, prevents shock wave-induced renal oxidative injury in rabbits. Urol Res 2006; 34: 277-282.
[29] Lv L, Wu SY, Wang GF, Zhang JJ, Pang JX, Liu ZQ, Xu W, Wu SG, Rao JJ. Effect of astragaloside IV on hepatic glucose-regulating enzymes in diabetic mice induced by a high-fat diet and streptozotocin. Phytother Res 2010; 24: 219-224.
[30] Tan S, Wang G, Guo Y, Gui D, Wang N. Preventive Effects of a Natural Anti-Inflammatory Agent, Astragaloside IV, on Ischemic Acute

Kidney Injury in Rats. Evid Based Complement Alternat Med 2013; 2013: 284025.
[31] Yang Y, Yue Y, Runwei Y, Guolin Z. Cytotoxic, apoptotic and antioxidant activity of the essential oil of Amomum tsao-ko. Bioresour Technol 2010; 101: 4205-4211.
[32] Resch M, Heilmann J, Steigel A, Bauer R. Further phenols and polyacetylenes from the rhizomes of Atractylodes lancea and their anti-inflammatory activity. Planta Med 2001; 67: 437-442.
[33] Wang L, Liu L, Shi Y, Cao H, Chaturvedi R, Calcutt MW, Hu T, Ren X, Wilson KT, Polk DB, Yan F. Berberine induces caspase-independent cell death in colon tumor cells through activation of apoptosis-inducing factor. PLoS One 2012; 7: e36418.
[34] Yin J, Xing H, Ye J. Efficacy of berberine in patients with type 2 diabetes mellitus. Metabolism 2008; 57: 712-717.
[35] Zhou L, Yang Y, Wang X, Liu S, Shang W, Yuan G, Li F, Tang J, Chen M, Chen J. Berberine stimulates glucose transport through a mechanism distinct from insulin. Metabolism 2007; 56: 405-412.
[36] Lu DY, Tang CH, Chen YH, Wei IH. Berberine suppresses neuroinflammatory responses through AMP-activated protein kinase activation in BV-2 microglia. J Cell Biochem 2010; 110: 697-705.
[37] Huang Z, Meng S, Wang L, Wang Y, Chen T, Wang C. Suppression of oxLDL-induced MMP-9 and EMMPRIN expression by berberine via inhibition of NF-κB activation in human THP-1 macrophages. Anat Rec (Hoboken) 2012; 295: 78-86.
[38] Wu D, Wen W, Qi CL, Zhao RX, Lü JH, Zhong CY, Chen YY. Ameliorative effect of berberine on renal damage in rats with diabetes induced by high-fat diet and streptozotocin. Phytomedicine 2012; 19: 712-718.
[39] Huang Z, Chen G, Shi P. Emodin-induced apoptosis in human breast cancer BCap-37 cells through the mitochondrial signaling pathway. Arch Pharm Res 2008; 31: 742-748.
[40] Liu Y, Jia L, Liu ZC, Zhang H, Zhang PJ, Wan Q, Wang R. Emodin ameliorates high-glucose induced mesangial p38 over-activation and hypocontractility via activation of PPARgamma. Exp Mol Med 2009; 41: 648-655.
[41] Wu F, Sun Y, Zhang T, Zhou QH, He LQ. A multicenter, randomized, open-label study of "Jianpi Qinghua Decoction" on Microinflammatory State in CKD2-3.Chinese Journal of Integrated Traditional and Western Nephrology 2012; 13: 504-506.
[42] Korn T, Oukka M, Kuchroo V, Bettelli E. Th17 cells: effector T cells with inflammatory properties. Semin Immunol 2007; 19: 362-371.
[43] Dong C. Diversification of T-helper-cell lineages: finding the family root of IL-17-producing cells. Nat Rev Immunol 2006; 6: 329-333.
[44] Polhill T, Zhang GY, Hu M, Sawyer A, Zhou JJ, Saito M, Webster KE, Wang Y, Wang Y, Grey ST, Sprent J, Harris DC, Alexander SI, Wang YM. IL-2/IL-2Ab complexes induce regulatory T cell expansion and protect against proteinuric CKD. J Am Soc Nephrol 2012; 23: 1303-1308.
[45] Mougiakakos D, Choudhury A, Lladser A, Kiessling R, Johansson CC. Regulatory T cells in cancer. Adv Cancer Res 2010; 107: 57-117.