ORIGINAL ARTICLE

Tumor necrosis factor-α blockade ameliorates diabetic nephropathy in rats

Dongsheng Cheng 1,*, Rulian Liang1,*, Baorui Huang2,*, Jiasheng Hou1,*, Jinyong Yin1, Ting Zhao1, Lu Zhou1, Rui Wu1, Youcun Qian1,3 and Feng Wang1

1Department of Nephrology, Shanghai Eighth People’s Hospital, Shanghai, China, 2Department of Emergency, Xiangya Hospital, Central South University, Changsha, China and 3Key Laboratory of Stem Cell Biology, CAS Center for Excellence in Molecular Cell Science, Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences & Shanghai Jiao Tong University School of Medicine, Shanghai, China

Correspondence to: Feng Wang; E-mail: zyzwq1030@hotmail.com

*These authors contributed equally to this work.

ABSTRACT

Background. Tubular injury plays a critical role in the development of diabetic nephropathy (DN), but current DN therapies do not combat tubular injury. This study was conducted to investigate if tumor necrosis factor (TNF)-α inhibition protects against tubular injury in diabetic rats and to examine the associated mechanisms.

Methods. Kidney biopsy tissues were collected and analyzed from 12 patients with DN and 5 control subjects. Streptozotocin (STZ)-induced diabetic rats were treated with a TNF-α inhibitor for 12 weeks. Renal function, albuminuria, histological injury, renal TNF-α messenger RNA (mRNA) and the NOD- (nucleotide-binding), LRR- (domain-like receptor) and pyrin domain-containing protein 3 (NLRP3) inflammasome were assessed.

Results. Diabetic patients with tubulointerstitial injury (TIN) presented with higher renal tubular expression of TNF-α mRNA and the NLRP3 inflammasome (P < 0.05). TNF-α inhibition reduced albuminuria, glomerular injury and tubular injury in STZ-induced diabetic rats (P < 0.05). Importantly, TNF-α inhibition significantly reduced the NLRP3 inflammasome in tubules (P < 0.05). Moreover, TNF-α inhibition decreased expression of tubular interleukin (IL)-6 and IL-17A mRNA.

Conclusions. TNF-α inhibition protects against TIN by suppressing the NLRP3 inflammasome in DN rats. Future studies may focus on the clinical protective effects of TNF-α inhibition using prospective observation.

Keywords: diabetic nephropathy, inflammasome, NLRP3, TNF-α, tubulointerstitial injury

INTRODUCTION

Diabetic nephropathy (DN) is the most common cause of end-stage renal disease (ESRD) in developed countries, with around one-third of diabetic patients developing nephropathy [1]. A better understanding of DN pathogenic mechanisms is direly needed to identify new therapeutic targets for DN. Although glomerulosclerosis is recognized as a primary feature of DN,
Tubular injury in DN is closely associated with increased risk of renal function decline [2]. Tubular injury can appear in early stages of DN and may have a crucial role in the early progression of DN [3]. Although the mechanism of tubular injury in diabetes is complex, it is known that key extracellular conditions contribute to damage in proximal tubules including hyperglycemia, proteinuria, hypoxia and inflammation [4, 5]. Tubular injury-related therapies could be an important targeted intervention for DN in the future.

It is well-recognized that NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome activation aggravates DN [6]. Moreover, the NLRP3 inflammasome plays a crucial role in tubular injury and renal fibrosis. NLRP3 deletion protects against renal fibrosis in mice models with 5/6 nephrectomy or unilateral ureteral obstruction [7, 8]. Additionally, high glucose induces NLRP3 inflammasome activation in HK-2 cells [9]. Thus, the NLRP3 inflammasome may be crucial to tubulointerstitial injury (TIN) development in DN.

Tumor necrosis factor (TNF)-α is a pleiotropic cytokine involved in inflammation induction, apoptotic cell death and immune modulation [10]. Moreover, TNF-α is an important transcriptional regulator of NLRP3 inflammasome components [11, 12]. Serum TNF-α and TNF receptors are increased in DN patients and are predictive of renal decline and incidence of ESRD [13–15]. Previous studies have reported that TNF-α inhibition could significantly alleviate diabetic glomerular lesions in diabetic rats [16, 17]. However, the protective effect of TNF-α inhibition on TIN in diabetes has not been investigated. In our preliminary study, diabetic patients with TIN presented with higher messenger RNA (mRNA) expression of TNF-α and NLRP3 inflammasome in renal tubules compared with DN patients without TIN. DN patients with TIN were defined as diabetic glomerular lesions with tubular–interstitial lesions scored more than 1. In the present study, we focused on whether TNF-α inhibition protected against renal TIN in diabetes and examined potential mechanisms. We hypothesized that TNF-α inhibition protects against TIN by suppressing the NLRP3 inflammasome in diabetic rats.

**MATERIALS AND METHODS**

**Human renal biopsies**

This study was approved by the ethics committee of Shanghai Eighth People’s Hospital. Kidney biopsy tissues were collected from 12 patients with DN and 5 control patients with histologically normal tissue surrounding carcinoma. The control patients did not have diabetes, hypertension or other kidney diseases. Informed consent was obtained from all subjects. Patient demographic data were recorded including age, ethnicity, gender and diabetes duration. Clinical laboratory values were recorded including hemoglobin, glycated hemoglobin, proteinuria and serum creatinine (SCR). TIN was evaluated using a semiquantitative scoring method [18, 19]. TIN was defined as tubular dilation and/or atrophy, tubular interstitial fibrosis or inflammatory cell infiltration. Briefly, TIN was graded from 0 to 3 as follows: 0, normal tubulointerstitium; 1, involving <25% of total area; 2, involving 25–50% of total area; and 3, involving >50% of total area.

According to renal histology appearance, DN patients were divided into two groups: DN group without TIN and DN with TIN group. DN without TIN was defined as diabetic glomerular lesion with few tubulointerstitial lesions scored less than 1. DN combined with TIN was defined as glomerular injury with tubulointerstitial lesions scored more than 1.

**Animal model**

Animal studies were approved by the Animal Care and Ethics Committee of Shanghai Jiao Tong University Affiliated Sixth People’s Hospital. All procedures were performed in accordance with the policies for the Care and Use of Laboratory Animals of our institution. Male Sprague–Dawley rats (weighing 250 ± 20 g) were provided by Shanghai Science Academy Animal Center (Shanghai, China). Diabetes was induced by an intraperitoneal (i.p.) injection of streptozotocin (a single dose: streptozotocin (STZ) 65 mg/kg; Sigma-Aldrich, St Louis, MO, USA). Diabetes was confirmed by blood glucose level >16.7 mM at 72 h following STZ injection. Rats were divided into four groups (n = 6 each): normal control group, rats treated with vehicle; STZ-induced diabetic rats treated with same volume of vehicle; STZ-induced diabetic rats with control immunoglobulin G (IgG) (Zhongkang Biotech, Hangzhou, China); and STZ-induced diabetic rats with Humira, a human anti-TNF monoclonal antibody (Abbott, Abbott Park, IL, USA) injected i.p. at 1.0 mg/kg/week. Humira treatment was initiated 7 days after STZ injection and continued weekly for 12 weeks. At the end of the experiments, a 24-h urine collection was conducted using metabolic cages. Blood and renal tissues were collected for analysis. The blood urea nitrogen (BUN), SCR and urine albumin were measured using enzyme-linked immunosorbent assay (ELISA) kits (Abcam, Cambridge, MA, USA). The ratio of kidney weight to body weight was calculated for the kidney weight index.

**Histological injury assessment**

The paraformaldehyde-fixed kidneys were embedded and then cut into 3 μm sections. Periodic acid–Schiff (PAS) staining was performed to assess histological alterations. As previously described [20], mesangial area was determined by the presence of PAS-positive and nuclei-free areas in the mesangium. Mesangial expansion index, expressed as the fraction of area of mesangial matrix area to glomerular area, was quantitatively measured using the software of ImageJ. The semiquantitative tubular injury scoring methods described above were used to assess TIN.

**Measurement of inflammation and oxidative stress markers**

Renal malondialdehyde (MDA) and superoxide dismutase (SOD) levels were measured using commercial kits according to the manufacturer’s protocol (Nanjing Jianchen, Nanjing, Jiangsu, China). Serum P-selectin and C-reactive protein (CRP) were measured using ELISA assay kits (Shanghai Immune Biotech, China). Renal caspase 1 activity was measured using a commercial colorimetric assay Kit (BioVision, Mountain View, CA, USA) following the manufacturer’s instructions. Renal interleukin (IL)-1β expression was measured using an ELISA kit (Invitrogen, Carlsbad, CA, USA).

**Western analysis and quantitative real-time PCR**

The glomeruli and tubulointerstitial tissues were isolated as previously described [21] and then stored at −80°C. Western blot was conducted as described previously [22]. The primary antibodies were anti-NLRP3 (Abcam) and anti-β-actin (Proteintech, Chicago, IL, USA). Quantitative real-time polymerase
chain reaction (PCR) was conducted as described previously [22]. The specific primers were as follows: human IL-1β: 5′ cccacccctttgttgag 3′ (forward) and 5′ ggccgcttaagttgtag 3′ (reverse); human NLRP3: 5′ agccccgtgctccatcctta 3′ (forward) and 5′ gccaagccacacactctct 3′ (reverse); human TNF-α: 5′ cccaggctc- caccccttc 3′ (forward) and 5′ gtccggatcatggcttgcag 3′ (reverse); rat IL-1β: 5′ gggccctgaaggggaagaa 3′ (forward) and 5′ agctg- caccctctc 3′ (reverse); rat IL-6: 5′ agccacacaggaacgaaagt 3′ (forward) and 5′ caaacatcagctcgaaggg 3′ (reverse); rat IL-17A: 5′ ctctgtgctgctgctactg 3′ (forward) and 5′ cctgctgttgctgacca 3′ (reverse); rat IL-18: 5′ aacagccaacgaatcccagac 3′ (forward) and 5′ ggtag- gactctctcactctctc 3′ (reverse); rat monocyte chemotactic protein 1: 5′ gaagtaagtgatcgcctggaact 3′ (forward) and 5′ taagggc- catcagcacagctgag 3′ (reverse); rat P-selectin: 5′ gcgtaccttggccttggct 3′ (forward) and 5′ gagggtgggtgtg 3′ (reverse); rat TNF-α: 5′ gagcccacccttggccttg 3′ (forward) and 5′ cctcggcgtttggacaca 3′ (reverse); rat NLRP3 inflammasome activation in TIN, NLRP3 inflammasome protein 1: 5′ ctagcatggccttttgtaac 3′ (forward) and 5′ agcccaccaggaacgaaagt 3′ (reverse); rat NLRP3 protein in tubules, as shown in Figure 6F and H, which significantly increased albuminuria (P < 0.05) (Figure 6A and B). Compared with the control group, treatment with TNF-α inhibition only decreased NLRP3 mRNA in both glomeruli and tubules (P < 0.05) (Figure 6E and G). However, TNF-α inhibition only decreased NLRP3 protein in tubules, as shown in Figure 6F and H, which

### Table 1. Characterization of patients with DN

| Group            | Ethnicity        | Gender (male/female) | Age (years) | SBP (mmHg) | DBP (mmHg) | HbA1c (%) |
|------------------|------------------|----------------------|-------------|------------|------------|-----------|
| Control          | Chinese, Han     | 3/2                  | 55.8 ± 4.1  | 133.6 ± 2.5| 79.8 ± 3.6 | 5.6 ± 0.4 |
| DN               | Chinese, Han     | 4/2                  | 58.5 ± 3.3  | 143.3 ± 4.8| 83.5 ± 5.3 | 7.3 ± 0.5  |
| DN with TIN      | Chinese, Han     | 4/2                  | 60.3 ± 3.5  | 147.8 ± 7.5| 86.8 ± 2.4 | 7.6 ± 0.4  |

Data were expressed as mean ± SEM. SBP, systolic blood pressure; DBP, diastolic blood pressure.

*P < 0.05 versus control group.

### Results

#### Renal Tubular TNF-α and NLRP3 Increased in Diabetic Patients with TIN

There are 12 subjects with DN and 5 control subjects enrolled in this study. According to the renal pathology, DN patients were divided into two groups: DN without TIN group (n = 6) and DN with TIN group (n = 6). The ethnicity of all the 17 subjects was Chinese Han. The basic clinical information is presented in Table 1. There were no significant differences in proteinuria between groups (Figure 1A). Scr was slightly elevated in diabetic patients with TIN compared with the DN group (Figure 1B).

Although DN combined with TIN group exhibited similar levels of mesangial expansion to the DN group, the tubular scores were significantly higher than that in the DN group (P < 0.05) (Figure 1C and D).

Compared with control subjects, all DN patients had higher renal TNF-α, NLRP3, and IL-1β mRNA expression in both glomeruli and tubules (Figure 2). Importantly, compared with DN patients (without TIN), renal tubular TNF-α, NLRP3 and IL-1β mRNA expressions were significantly increased in the TIN group, while there was no difference in glomeruli (P < 0.05) (Figure 2).

Thus, diabetic patients with TIN presented with higher renal tubular expression of TNF-α mRNA and NLRP3 inflammasome.

#### TNF-α Inhibition Reduces Renal Injury in DN Rats

A STZ-induced DN rat model was used to assess the effects of TNF-α inhibition and examine the underlying mechanisms. STZ-induced DN rats had increased blood glucose, HbA1c, Scr, BUN, kidney weight index and albuminuria and reduced body weight at 12 weeks compared with the control group (Figures 3 and 4). TNF-α inhibition had no effect on blood glucose and HbA1c levels (Figure 3A and B). Additionally, there was no significant difference in BUN and Scr between the IgG control antibody-treated DN group and the TNF-α inhibition treatment group (Figure 3C and D). Compared with STZ-induced rats + IgG group, treatment with TNF-α inhibition decreased kidney weight index (P < 0.05) (Figure 4B). Moreover, TNF-α inhibition significantly decreased albuminuria (P < 0.05) (Figure 4C).

Consistent with the biochemistry data, TNF-α inhibition reduced mesangial expansion, which was a measure of histological glomerular injury in DN rats (P < 0.05) (Figure 4D). Importantly, TNF-α inhibition significantly attenuated tubular injury (P < 0.05) (Figure 4E). Therefore, TNF-α inhibition could alleviate both histologic glomerular injury and tubular injury in DN rats.

#### TNF-α Inhibition Alleviated Renal Inflammation and Oxidative Stress in DN Rats

Inflammatory response and oxidative stress are involved in the pathogenesis of DN. As shown in Figure 5, compared with STZ-induced rats + IgG group, TNF-α inhibition significantly reduced serum CRP (Figure 5A). Meanwhile, TNF-α inhibition decreased TGF-β and P-selectin in both serum and renal cortex (Figure 5B, C and F). TNF-α inhibition decreased renal MDA levels and increased renal SOD levels (Figure 5D and E). Therefore, TNF-α inhibition attenuates kidney injury in diabetic rats in part through suppression of inflammation activation and oxidative stress.

#### TNF-α Inhibition Attenuated Renal NLRP3 Inflammasome in DN Rats

To determine the association between TNF-α pathway and NLRP3 inflammasome activation in TIN, NLRP3 inflammasome in both glomeruli and tubules was separately measured in this study. As shown in Figures 6 and 7, compared with the control group, STZ-induced DN rats had increased renal caspase-1 activity and renal IL-1β expression (P < 0.05) (Figure 6A and B) as well as increased NLRP3, IL-1β and IL-18 mRNA in both glomeruli and tubules (Figures 6C–E, 7C and F). Compared with the vehicle treatment DN group, TNF-α inhibition significantly reduced NLRP3 mRNA in both glomeruli and tubules (P < 0.05) (Figure 6E and G). However, TNF-α inhibition only decreased NLRP3 protein in tubules, as shown in Figure 6F and H, which...
suggested that the effects of TNF-α inhibition on NLRP3 might be stronger in renal tubules than glomeruli. Our previous study reported that inhibition of IL-6 and IL-17A could suppress NLRP3 inflammasome activation [22], thus IL-6 and IL-17A mRNA expression in both glomeruli and tubules was further investigated. As expected, TNF-α inhibition decreased IL-6 and IL-17A mRNA expression in both renal glomeruli and tubules (Figure 7A, B, D and E). Therefore, TNF-α inhibition protects against renal TIN in diabetic rats partly through suppression of NLRP3 inflammasome activation by inhibiting IL-6 and IL-17A.

DISCUSSION
This study demonstrated that TNF-α inhibition protected against renal TIN in diabetic rats and provided underlying molecular mechanisms for the protective properties of TNF-α inhibition. Diabetic patients with TIN presented with higher renal tubular expression TNF-α and NLRP3 inflammasome. TNF-α inhibition reduced albuminuria, histologic glomerular injury and tubular injury in DN rats. Furthermore, TNF-α inhibition may protect against tubular injury by suppressing the NLRP3 inflammasome and decreasing oxidative stress.

Tubular injury plays a critical role in the development of DN and is closely related with renal prognosis in diabetes. Although renin-angiotensin-aldosterone system (RASS) inhibitors are considered the standard intervention for DN patients, the protective effect of RASS inhibition on tubular injury is unknown. Treatment with the angiotensin-converting enzyme inhibitor enalapril could reduce albuminuria in 16-week-old db/db mice, but does not ameliorate tubular damage [23]. New therapies to diminish tubular injury in DN are urgently needed.
Although the pathogenesis of tubular injury in DN is unclear, it is known that inflammation is involved in tubular damage [2, 3, 5]. TNF-α is a pleiotropic cytokine that activates TNF receptors, leading to the activation of pro-inflammatory mediators, apoptosis and necroptosis [10]. This cytokine is expressed, synthesized and released by infiltrating and resident cells of the kidney [24]. In the current study, TNF-α inhibition decreased albuminuria and protected from diabetic tubulopathy in STZ-induced diabetic rats. These beneficial effects of TNF-α inhibition might be partly associated with suppression of NLRP3 inflammasome activation by inhibiting IL-6 and IL-17A. This hypothesis is supported by the following findings: first, TNF-α inhibition decreased renal IL-6, IL-17A and NLRP3 inflammasome expression in the present study. Moreover, the NLRP3 inflammasome in both glomeruli and tubules was separately measured; the results demonstrated that TNF-α inhibition significantly reduced NLRP3 inflammasome in tubules. Second, the NLRP3 inflammasome plays a critical role in the pathogenesis and progression of DN, whereas NLRP3 deficiency ameliorates tubular injury in multiple types of renal disease [7, 8].
Third, the presence of IL-17A activates the NLRP3 inflammasome, while neutralization of IL-17A decreases the NLRP3 inflammasome expression [25–27]. Importantly, IL-6 promotes the differentiation of T helper 17 cells, which secrete IL-17, whereas blockade of IL-6 receptor significantly reduced renal NLRP3 inflammasome and IL-17A in diabetic rats [21, 28, 29].

These findings support a crucial role of TNF-α in tubular injury. Several studies should be conducted in the future. TNF-α inhibitors are widely used biochemical drugs in clinical settings, especially in rheumatoid disease, and are considered safe for long-term treatment. TNF-α inhibition may be a viable treatment option for TIN in DN if future clinical studies confirm these protective effects. Moreover, there are different types of TNF-α inhibitors, such as chimeric monoclonal antibody, soluble TNF-α receptor fusion protein and pentoxifylline. These drugs should be tested separately.

There were some limitations in the current study. First, the human renal biopsies sample size was small. Second, streptozotocin-induced type 1 diabetic rats were used in animal experiments. In the future, the effect of TNF-α inhibition on TIN needs been further investigated in type 2 diabetic animal models. In addition, most data were based on mRNA expression...
level, thus changes on proteomic level were expected and the molecular mechanism should be more deeply investigated in further studies. Third, TNF receptors are an important part in the TNF pathway, thus future study is needed to determine the direct effect of TNF receptors on TIN in DN.

**CONCLUSION**

This study concludes that TNF-α inhibition can protect against TIN in diabetic rats through suppression of the NLRP3 inflammasome. TNF-α inhibition may be a promising new therapy for the treatment of TIN in DN. Future studies should focus on the clinical protective effect of TNF-α inhibition in prospective clinical trials.

**ACKNOWLEDGEMENTS**

Thank you to Dr Chun Yang from the Medical College of Wisconsin for technical support.

**FUNDING**

This work was sponsored by the National Natural Science Foundation of China (81570603 and 81770741) and the
Health and Family Planning Commission of Shanghai Xuhui District (SHXH201605).

AUTHORS’ CONTRIBUTIONS
F.W. designed the experiments. D.C., R.L., B.H., J.Y., T.Z., L.Z., R.W. and F.W. performed the experiments. F.W. and Y.Q. analyzed and interpreted the data. D.C., B.H. and F.W. drafted the manuscript.

CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest. The results presented in this paper have not been published previously, in whole or part, except in abstract format.

REFERENCES
1. Saran R, Robinson B, Abbott KC et al. US renal data system 2017 annual data report: epidemiology of kidney disease in the United States. Am J Kidney Dis 2018; 71: A7
2. Gilbert RE. Proximal tubulopathy: prime mover and key therapeutic target in diabetic kidney disease. Diabetes 2017; 66: 791–800
3. Bonventre JV. Can we target tubular damage to prevent renal function decline in diabetes? Semin Nephrol 2012; 32: 452–462
4. Lin M, Yiu WH, Wu HJ et al. Toll-like receptor 4 promotes tubular inflammation in diabetic nephropathy. J Am Soc Nephrol 2012; 23: 86–102
5. Zeni L, Norden AGW, Cancarini G et al. A more tubulocentric view of diabetic kidney disease. J Nephrol 2017; 30: 701–717
6. Qi Y, Tang LQ. Roles of the NLRP3 inflammasome in the pathogenesis of diabetic nephropathy. Pharmacol Res 2016; 114: 251–264
7. Gong W, Mao S, Yu J et al. NLRP3 deletion protects against renal fibrosis and attenuates mitochondrial abnormality in mouse with 5/6 nephrectomy. Am J Physiol Renal Physiol 2016; 310: F1081–F1088
8. Vilaysane A, Chun J, Seamone ME et al. The NLRP3 inflammasome promotes renal inflammation and contributes to CKD. J Am Soc Nephrol 2010; 21: 1732–1744
9. Chen K, Zhang J, Zhang W et al. ATP-P2X4 signaling mediates NLRP3 inflammasome activation: a novel pathway of diabetic nephropathy. Int J Biochem Cell Biol 2013; 45: 932–943
10. Vassalli P. The pathophysiology of tumor necrosis factors. Annu Rev Immunol 1992; 10: 411–452
11. McGeough MD, Wree A, Inzaugarat ME et al. TNF regulates transcription of NLRP3 inflammasome components and inflammatory molecules in cryopyrinopathies. J Clin Invest 2017; 127: 4488–4497
12. Amaral FA, Bastos LF, Oliveira TH et al. Transmembrane TNF-alpha is sufficient for articular inflammation and hypernociception in a mouse model of gout. Eur J Immunol 2016; 46: 204–211
13. Gohda T, Niewczas MA, Ficociello LH et al. Circulating TNF receptors 1 and 2 predict stage 3 CKD in type 1 diabetes. J Am Soc Nephrol 2012; 23: 516–524
14. Niewczas MA, Gohda T, Skupien J et al. Circulating TNF receptors 1 and 2 predict ESRD in type 2 diabetes. J Am Soc Nephrol 2012; 23: 507–515
15. Coca SG, Nadkarni GN, Huang Y et al. Plasma biomarkers and kidney function decline in early and established diabetic kidney disease. J Am Soc Nephrol 2017; 28: 2786–2793
16. Omote K, Gohda T, Murakoshi M et al. Role of the TNF pathway in the progression of diabetic nephropathy in KK-A(y) mice. Am J Physiol Renal Physiol 2014; 306: F1335–F1347
17. Awad AS, You H, Gao T et al. Macrophage-derived tumor necrosis factor-alpha mediates diabetic renal injury. Kidney Int 2015; 88: 722–733
18. Radford MG Jr, Donadio JV Jr, Bergstralh EJ et al. Predicting renal outcome in IgA nephropathy. J Am Soc Nephrol 1997; 8: 199–207.
19. Angelotti ML, Ronconi E, Ballerini L et al. Characterization of renal progenitors committed toward tubular lineage and their regenerative potential in renal tubular injury. Stem Cells 2012; 30: 1714–1725
20. Lin Y, Sun Z. Thyroid hormone ameliorates diabetic nephropathy in a mouse model of type II diabetes. J Endocrinol 2011; 209: 185–191
21. Richardson JC, Waterson P, Simmons NL. Isolation and culture of renal cortical tubules from neonate rabbit kidneys. Q J Exp Physiol 1982; 67: 287–301
22. Wu R, Liu X, Yin J et al. IL-6 receptor blockade ameliorates diabetic nephropathy via inhibiting inflammasome in mice. Metabolism 2018; 83: 18–24
23. Marquardt A, Al-Dabet MM, Gosh S et al. Farnesoid X receptor agonism protects against diabetic tubulopathy: potential add-on therapy for diabetic nephropathy. J Am Soc Nephrol 2017; 28: 3182–3189
24. Al-Lamki RS, Mayadas TN. TNF receptors: signaling pathways and contribution to renal dysfunction. Kidney Int 2015; 87: 281–296
25. Kim SR, Kim HJ, Kim DI et al. Blockade of interleukin-17A and endoplasmic reticulum stress attenuates LPS-induced lung injury. Theranostics 2015; 5: 1343–1362
26. Yan J, Li Y, Yang H et al. Interleukin-17A participates in podocyte injury by inducing IL-1beta secretion through ROS-NLRP3 inflammasome-caspase-1 pathway. Scand J Immunol 2018; 87: e12645
27. Zhang S, Yu N, Zhang R et al. Interleukin-17A induces IL-1beta secretion from RPE cells via the NLRP3 inflammasome. Invest Ophthalmol Vis Sci 2016; 57: 312–319
28. Kimura A, Kishimoto T. IL-6: regulator of Treg/Th17 balance. Eur J Immunol 2011; 57: 1830–1835
29. Serada S, Fujimoto M, Mihara M et al. IL-6 blockade inhibits the induction of myelin antigen-specific Th17 cells and Th1 cells in experimental autoimmune encephalomyelitis. Proc Natl Acad Sci USA 2008; 105: 9041–9046