IL-17C neutralization protects the kidney against acute injury and chronic injury

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Summary

Background Interleukin-17C (IL-17C), a member of the IL-17 cytokine family, plays a pathogenic role in kidney diseases. Our previous studies have shown that pre-administration of IL-17C neutralizing antibody attenuated acute kidney injury (AKI, a common acute inflammation associated renal disease). In this study, we explored whether post-ischemia reperfusion (IR) of IL-17C blockade has therapeutic effects on AKI and whether IL-17C is involved in the pathogenesis of diabetic nephropathy (DN), a major type of chronic inflammation-associated kidney disease.

Methods 12-week-old male C57BL/6JGpt mice were treated with IL-17C neutralizing antibody or normal IgG control antibody at 3 h after reperfusion. Renal injury, inflammation, and oxidative stress were assessed. Additionally, we examined renal IL-17C expression in patients with DN and *db/db* mice and evaluated albuminuria, mesangial matrix accumulation and podocyte loss in *db/db* mice with IL-17C neutralization. Knockdown of NF-κB p65 using siRNA, and blocking Hypoxia-inducible factor-1α (HIF-1α) using YC-1 in mice and HIF-1α Decoy in HK2 cells were investigated to explore the possible signaling pathway involved in IL-17C regulation.

Findings We found that delayed IL-17C neutralization had similar reno-protective effects on renal ischemia-reperfusion injury (IRI). Additionally, renal IL-17C expression was increased in patients with DN and *db/db* mice, while IL-17C blockade significantly attenuated DN, accompanied with blunted albuminuria, mesangial matrix accumulation, and podocyte loss. Moreover, IL-17C neutralization significantly repressed the expression of downstream pro-inflammatory cytokines, inflammatory cell infiltration, and Th17/IL-17A activation both in mice with renal IRI and DN. Mechanistical studies demonstrated that hypoxia or high glucose-induced IL-17C up-regulation was predominantly mediated by NF-κB pathway.

Interpretation IL-17C participates in the pathogenesis of AKI and DN and inhibition of IL-17C shows potential as a therapeutic strategy for AKI and DN.

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Keywords: Acute kidney injury; Ischemia/reperfusion injury; Diabetic nephropathy; IL-17C

Introduction

Interleukin-17C (IL-17C), a member of the IL-17 cytokine family, was cloned from a human fetal kidney library in 2000 from a large-scale homology search based on the sequence of IL-17A.[1] In contrast to other members of the IL-17 family, IL-17C is expressed primarily by epithelial cells in response to pro-inflammatory cytokines (TNF-α and IL-1β) or microorganisms.[2,3] IL-17RE, the specific receptor of IL-17C, is preferentially expressed on Th17 and epithelial cells.[2–4]

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Research in context

Evidence before this study

Inflammation is critically involved in the pathogenesis of kidney disease both in acute and chronic kidney diseases. It has been established that IL-17C plays a crucial role in inflammation-mediated tissue injury. Our previous study has reported that IL-17C plays a pathogenic role in AKI and pre-administration of IL-17C neutralizing antibody attenuated renal dysfunction. However, post-ischemia reperfusion (IR) of IL-17C neutralization has more clinical significance for AKI therapy. Besides, whether IL-17C is involved in the pathogenesis of diabetic nephropathy (DN), a major type of chronic inflammation-associated kidney disease, remains unknown.

Added value of this study

Clinical therapeutic approaches to prevent renal injury in patients with AKI and diabetes are limited. In this study, we confirmed the therapeutic effects of post-IR of IL-17C blockade in AKI mice. We also found that IL-17C was up-regulated both in diabetes patients and mice, while IL-17C blockade effectively delayed renal injury development in *db/db* mice. Expression of downstream pro-inflammatory cytokines, inflammatory cell infiltration, and Th17/IL-17A activation were significantly inhibited in mice treated with IL-17C neutralizing antibody. Mechanistical studies demonstrated that hypoxia or high glucose-induced IL-17C up-regulation was predominantly mediated by NF-κB pathway instead of Hypoxia-inducible factor-1α (HIF-1α) pathway.

Implications of all the available evidence

Our study demonstrated that hypoxia and hyperglycemia both could induce IL-17C up-regulation. Blockade of IL-17C had protective effects in AKI and DN, providing insights into the pathogenesis of inflammation-associated kidney diseases and possible clinical strategies for AKI and DN. Additionally, our data indicated that IL-17C was up-regulated via NF-κB pathway in kidneys *in vivo* and HK2 cells *in vitro*. In contrast, HIF-1α was not involved in the induction of IL-17C. These results indicated that IL-17C may be a potential target for patients with AKI or DN.

On one hand, IL-17C signals through IL-17RE to activate Th17/IL-17A response, which may mediate tissue injury through neutrophil recruitment in several autoimmune diseases, including experimental crescentic glomerulonephritis, psoriasis, experimental autoimmune encephalomyelitis.[2,4,5] On the other hand, IL-17C/IL-17RE axis directly induces the expression of pro-inflammatory chemokines, cytokines, and antimicrobial peptides involved in mucosal host defense or inflammatory amplification from epithelial cells, and ultimately resulted in tissue damage in an autocrine manner.[2,3,6,7] Thus, IL-17C plays a crucial role in mucosal host defense and inflammation mediated tissue injury.

Evidence indicates that IL-17C signaling pathway is critically involved in inflammation-mediated kidney injury. Previous studies showed that IL-17RE was highly expressed in the kidney and IL-17RE deficiency significantly decreased IL-17C mediated up-regulation of pro-inflammatory cytokines, chemokines, and antimicrobial peptides.[3] IL-17C expression was highly induced in kidney epithelial cells after Candida albicans infection, whereas inhibition of IL-17C resulted in attenuated kidney inflammation and increased survival in systemic fungal infection.[8] Moreover, it has been reported that IL-17C/IL-17RE signaling mediated tissue injury via Th17 activation and subsequently neutrophil recruitment in experimental models of crescentic glomerulonephritis.[5] Collectively, these data demonstrate that activation of IL-17C/IL-17RE signaling pathway can lead to renal injury, particularly within the context of inflammation.

AKI (a common acute inflammation-associated renal disease) and diabetic nephropathy (DN, a chronic inflammation-associated renal disease) are two major types of kidney diseases with a high prevalence worldwide.[9–12] Despite tremendous scientific and clinical efforts, there is still a lack of effective prophylactic or therapeutic interventions to prevent renal injury and the decline of renal function in patients with AKI and DN. There is also a lack of specific therapeutic approaches to reverse them.[13] Our previous studies have found that pre-treatment of IL-17C neutralizing antibody mitigated renal ischemia-reperfusion injury (IRI).[14] However, occurrence of AKI is often clinically unpredictable, so therapeutic measures that can reverse or ameliorate renal dysfunction after injury are of more significance in clinical practice. Thus, we further investigated whether delayed IL-17C neutralization could protect against renal IRI in this study. Additionally, *db/db* mice, as one of the representative models of chronic inflammation-mediated renal injury, were used to determine the pathogenic role of IL-17C in DN. The underlying molecular mechanisms involved in the regulation of IL-17C gene expression under AKI and diabetes were also investigated *in vivo* and *in vitro*.

Methods

Human kidney biopsy samples

Subjects diagnosed with DN, without any other type of renal injury and control subjects diagnosed with minimal injury or no obvious kidney pathology were enrolled and clinical data were recorded in the study. The diagnostic criteria of diabetic kidney disease followed the KDIGO guidelines. Patients with infectious disease, psoriasis, autoimmune encephalomyelitis,

nephritis, acute kidney injury, and dialysis were excluded. The kidney biopsy blocks were cut into 3 μm sections. Total RNA was extracted from sections of each renal biopsy specimens for RT-qPCR as described previously.[15]

Ethics

Protocols involving the use of human medical information and kidney biopsy samples were approved by the Ethics Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital. The study was conducted in accordance with the World Medical Association Declaration of Helsinki. The participants were randomly chosen from patients hospitalized from January 2016 to September 2018 in Shanghai Jiao Tong University Affiliated Sixth People's Hospital. Written informed consent was obtained from each participant.

All animal experiments and procedures were approved by the Institutional Animal Care and Use Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital (license number: SYXK 2021-0028) and conformed to the regulatory standards in accordance with the National Institutes of Health Guide (Guide for the Care and Use of Laboratory Animals, 2011).

Animals and study design

C57BL/6JGpt (RRID: IMSR_GPT:N000013) male mice and male *db/db* (RRID: IMSR_GPT:T002407) mice were provided by Nanjing Biomedical Research Institute of Nanjing University.

Study 1 was performed to evaluate the reno-protection of post-IR IL-17C neutralization

Twelve weeks old male C57BL/6JGpt mice were randomly divided into three groups (n = 6 for each): 1) IRI + IL-17C antibody group: mice were treated with IL-17C polyclonal antibody CIGBIO Biomed (Hangzhou, China) at a dose of 10 mg/kg via tail vein injection 3 h after reperfusion; 2) IRI + IgG group: mice treated with normal IgG control antibody at a similar dose to IL-17C antibody group; 3) Sham group. Bilateral renal ischemia was induced by non-traumatic microvascular clamps over the renal pedicles for 30 min, and then the clamps were removed to start reperfusion. Sham-operated mice were subjected to exposure of renal pedicels. All the mice were sacrificed at 24 h after reperfusion and tissues were harvested as previously described.[14]

Study 2 was performed to examine the reno-protective effects of IL-17C neutralization in db/db *mice*

Twelve weeks old male *db/db* mice and age-matched *db/m* littermates were randomly allocated to assigned groups (n = 6 for each). IL-17C neutralization antibody or control antibody was administered at 10 mg/kg twice a week via tail vein injection. All animals were housed in metabolic cages to collect 24-h urine samples and then sacrificed to harvest blood and kidney tissues at 20 weeks.

Study 3 was performed to identify the signaling pathways involved in the regulation of IL-17C expression in vivo

Firstly, to determine whether NF-κB pathways are involved in IL-17C regulation under AKI, 12-week-old male C57BL/6JGpt mice were randomly allocated to sham group, ischemia + scrambled siRNA group, and ischemia + p65 siRNA group (n = 6 for each). Secondly, 12-week-old male C57BL/6JGpt mice were randomly divided into sham group, ischemia + Vehicle group, and ischemia + YC-1 group (n = 6 for each) to determine whether HIF-1α pathways is involved in IL-17C regulation undergoing renal ischemia. Lastly, 20-week-old male *db/db* mice and age-matched *db/m* littermates were randomly allocated to db/m + scrambled siRNA group, db/m + p65 siRNA group, *db/db* + scrambled siRNA group, and *db/db* + p65 siRNA group (n = 6 for each) to explore the role of NF-κB pathways in IL-17C regulation in diabetes. NF-κB p65 siRNA and YC-1, a specific inhibitor of HIF-1α were used to examine the changes of IL-17C expression in IRI mice, respectively. Briefly, scrambled siRNA or p65 siRNA (Ribobio, Guangzhou, China, 10 nmol/d) was injected intraperitoneally for three successive days before ischemia, while YC-1 (Sigma-Aldrich, St Louis, MO, USA, 2 mg/kg) was intraperitoneally injected 24 h before ischemia[16]. All the mice were sacrificed 30 min after ischemia and kidney tissues were harvested. In addition, p65 siRNA was administered for three successive days in *db/db* mice at 20 weeks of age before mice were sacrificed for tissues collection.

Cell culture

Human proximal tubular epithelial cells (HK2; ATCC, Old Town Manassas, USA, RRID: CVCL_0302) were cultured in keratinocyte serum-free medium (17005-042; Gibco), supplemented with 0.05 mg/ml bovine pituitary extract and 5 ng/ml human recombinant EGF. The HK2 cell lines were identified with STR markers by Beyotime Institute of Biotechnology. The HIF-1α Decoy and CoCl2 were provided by ShineGene Molecular Biotech (Shanghai, China) and Sigma-Aldrich (St. Louis, MO, USA), respectively. For hypoxia induction, HK2 cells were incubated under hypoxia condition (1% O_2, 94% N_2, and 5% CO_2) or normoxia condition (20% O_2, 5% CO_2) for 6 h. As for high glucose induction, HK2 cells were incubated in solutions with different concentrations of glucose for 24 h: 5 mM (normal glucose, Control group), 24.5 mM mannitol with 5.5 mM glucose (high mannitol, Mannitol group) or 30 mM (high glucose, HG group). IL-17C or p65 were silenced using small interfering RNAs in HK2 cells. HIF-1α Decoy was administrated to block HIF-1α under hypoxia condition, while $CoCl_2$ was used to stabilize HIF-1α expression under normoxic condition. IL-17C siRNA, p65 siRNA,

and HIF-1α Decoy were pre-treated 24 h before hypoxia, high glucose induction or cell collection. All HK2 cells and culture supernatants were collected.

Real-time quantitative polymerase chain reaction

Quantitative polymerase chain reaction was performed as described previously.[14] Briefly, total RNA was extracted from kidney tissues or HK2 cells using Trizol reagent (Invitrogen, Grand Island, NY, USA) following the manufacturer's instructions. 18S ribosomal RNA was used as the internal reference gene. All the primer sequences are listed in Table 1.

Fluorescence-activated cell sorting (FACS) analysis

FACS analysis was performed as previously.[17] Briefly, freshly harvested kidneys were harvested and digested in liberase (2 μg/ml; Roche) for 15 min at 37 °C using Gentle MACs (Miltenyi, German). The digested tissue was filtered through a 100-μm mesh and washed with RPMI containing 10% fetal bovine serum (Invitrogen). Cells were scanned using flow cytometry (Fortessa X20, BD Biosciences, USA), and data were analyzed using FlowJo software (Tree Star, Inc.). The primary antibodies were as follows: Brilliant Violet 786™ rat anti-IL-17A monoclonal antibody (eBio17B7, eBioscience, RRID: AB_2925749), FITC rat anti-FOXP3 monoclonal antibody (FJK-16s, eBioscience, AB_465243), and Brilliant Violet 421™ rat anti-CD4 antibody (GK1.5, BioLegend, RRID: AB_10900241).

Western blot

Western blot was conducted as described previously.[14] The primary antibodies were rabbit anti-IL-17C antibody (#PA5-79474, Invitrogen, RRID: AB_2746590) and mouse anti-α-Tubulin (#66031-I-Ig, Proteintech, RRID: AB_11042766). IL-17C levels were normalized with α-Tubulin. Secondary antibody was from Invitrogen (goat anti-rabbit IgG-HRP, #UH283636; goat anti-mouse IgG-HRP, #UB278606).

Species	Gene	Forward sequence (5′ to 3′)	Reverse sequence (5′ to 3′)
Human	IL-17C	CCCTCAGCTACGACCCAGT	CTTCTGTGGATAGCGGTCCT
Human	IL-17RE	ACTGTTCCCGCTGTTTGTGC	GGCCCCGTTGAAGACCTG
Human	IL-6	ACTCACCTCTTCAGAACGAATTG	CCATCTTTGGAAGGTTCAGGTTG
Human	IL-1β	CCCCAGCCCTTTTGTTGAG	GGCGGGCTTTAAGTGAGTAGG
Human	Renalase	GAAAAATCATTGCAGCCTCTCA	AAGTTCTGCCTGTGCCTGTGTA
Mouse	IL-17C	CCTCTAGCTGGAACACAGTGC	GCGGTTCTCATCTGTGTCG
Mouse	IL-17RE	GAGGCCAACGCACCAAAAG	CACCTCGGGCAGCAAATC
Mouse	IL-6	GATGGATGCTACCAAACTGGAT	CCAGGTAGCTATGGTACTCCAGA
Mouse	IL-1β	TGTAATGAAAGACGGCACACC	TCTTCTTTGGGTATTGCTTGG
Mouse	TNF-α	TCTTCTCATTCCTGCTTGTGG	GGTCTGGGCCATAGAACTGA
Mouse	IL-17A	GAGAGCTGCCCCTTCACTTTC	AGCCGCGGGTCTCTGTTTA
Mouse	MCP-1	GAATGTGAAGTTGACCCGTAAATCT	TAAGGCATCACAGTCCGAGTCA
Mouse	NF-κB p65	CACCAAGGATCCACCTCACC	CTCTATAGGAACTATGGATACTGCG
Mouse	KIM-1	CCTTGTGAGCACCGTGGCTA	TGTTGTCTTCAGCTCGGGAATG
Mouse	NGAL	ACCACGGACTACAACCAGTTC	AAGCGGGTGAAACGTTCCTT
	18S rRNA	CGGCTACCACATCCAAGGAA	CCTGTATTGTTATTTTTCGTCACTACCT

Table 1: **The primers for real-time quantitative PCR.**

Biochemical measurements

An automatic biochemical analyzer (7600; Hitachi, Tokyo, Japan) was used to measure blood urine nitrogen and serum creatinine. Renal NF-κB activity was determined with an ELISA kit targeting phosphorylated Ser536 (Abcam, Cambridge, USA). Commercial kits (Nanjing Jiancheng Bio-Engineering Institute, Nanjing, Jiangsu, China) were used to measure MDA and SOD as previously described,[14] and the SOD kits detects only Mn-SOD isoforms. Blood glucose levels were determined using a glucose meter (Roche, Basel, Switzerland). Hemoglobin A1c (HbA1c) was measured using an A1cNOW kit (Bayer, Leverkusen, Germany). Fasting plasma insulin concentrations were measured by an ultrasensitive mouse insulin ELISA kit (Mercodia, Uppsala, Sweden). 24-h urine collections were harvested from mice using metabolic cages and then measured with enzyme linked immunosorbent assay (ELISA) kits (Abcam, Cambridge, USA) for the evaluation of urine albumin.

Commercial ELISA kits were used to measure the protein levels of IL-17C, IL-6, and IL-1β in supernatants of HK2 cells under high glucose or hypoxia (IL-17C Elisa kits were purchased from CIGBIO Biomed (Hangzhou, China); IL-6, and IL-1β were purchased from Arigo Biolaboratories, Shanghai, China).

Histological analysis, immunohistochemistry, and immunofluorescence

Kidney histological analysis was conducted as previously described.[14] Briefly, each formalin-fixed kidney was embedded in paraffin and cut into 3 μm sections for further analysis. After periodic acid–Schiff staining (PAS) staining, the slides were viewed by light microscopy. Tubular injury was assessed by a semi-quantitative scoring system that graded the percentage of tubular necrosis, loss of brush border, tubular dilatation, and cast formation in randomly chosen fields. The degree of injury was estimated by the following criteria: 0, none; 1, 0–10%; 2, 11–25%; 3, 26–45%; 4, 46–75%; and 5, 76–100%, as described previously.[14] The mesangial matrix areas identified by the presence of PAS-positive and nuclei-free areas in the glomeruli were quantitatively analyzed by Image J software based on an average of 30 glomeruli per mouse. Matrix index was calculated as the ratio of mesangial area to tuft area.[18] The ratio of kidney weight to bodyweight was considered as kidney weight index.

IL-17C expression was assessed in the kidney using immunohistochemistry and *in situ* hybridization as described previously.[14,19] Podocytes, monocytes/macrophages, and neutrophils cell were detected by immunofluorescence with Wilms tumor protein 1 (WT-1,

#ab89901, Abcam, RRID: AB_2043201), F4/80 (#ab6640, Abcam, RRID: AB_1140040), myeloperoxidase (MPO, #ab208670, Abcam, RRID: AB_2864724), respectively. Assessment of podocyte numbers were calculated as previously described.[20]

Statistical analysis

SPSS statistical software (Version 19.0; Chicago, IL, USA) was used for data processing. All data were expressed as mean ± standard error of the mean (SEM). Variance was analyzed for significance using the student's t test for comparisons between 2 groups or using ANOVA followed by Holm–Šidák post hoc tests for more than 3 groups. Shapiro–Wilk's test confirmed that all variable data in accordance with the law of normal distribution (all $p > 0.05$). A value of $p < 0.05$ was considered significant.

Role of funding source

The funders had no role in the study design, data collection, data analyses, interpretation, or writing of report, and the decision of paper submission.

Results

Post-IR treatment of IL-17C neutralizing antibody effectively attenuated renal IRI in mice

Our previous studies have shown that pre-treatment with IL-17C neutralizing antibody prevented against renal IRI in mice.[14] To further investigate whether post-IR administration of IL-17C neutralizing antibody could exert similar reno-protective effects in AKI, IL-17C neutralizing antibody or normal control IgG (10 mg/kg) were administered after reperfusion via tail vein injection. Mice with 30 min of bilateral ischemia manifested significant kidney injury within 3 h of reperfusion, as indicated by previously established AKI biomarkers, NGAL, and KIM-1. Our results showed significant elevation of serum NGAL, urine KIM-1, and renal NGAL and KIM-1 mRNA occurred at 3 h after ischemic injury compared with sham-operated mice and persisted for at least 24 h (Fig. S1a–d). IL-17C was initially induced at 3 h after reperfusion and reached its peak level at 6 h. Consequently, we chose to deliver IL-17C neutralizing antibody at 3 h after reperfusion (Fig. S1e). In agreement with the results of IL-17C antibody pre-administered experiments, post-IR administration of IL-17C antibody significantly attenuated IR-induced elevation of blood urine nitrogen and serum creatinine (Fig. 1a and b). Furthermore, renal ischemia/reperfusion led to increased levels of malondialdehyde (MDA) and decreased activity of superoxide dismutase (SOD). This was reversed by IL-17C neutralization after IRI (Fig. 1c and d). Histological analysis demonstrated that the renal tubular detachment, brush border loss, necrosis of tubular cells, as well as tubular injury scores were significantly attenuated in IL-17C antibody post-IR-administered IRI group compared with IRI + IgG control group (Fig. 1e and f). Taken together, these data indicated that post-IR IL-17C neutralization had therapeutic effects against AKI.

Expression of pro-inflammatory cytokines, Th17 cells activation, and renal inflammatory cells infiltration were blunted by delayed IL-17C neutralization in IRI mice

It has been established that the pro-inflammatory cytokines TNFα, IL-6, and IL-1β are downstream targets of IL-17C signaling and IL-17C can mediate renal inflammation via activation of Th17/IL-17A axis.[5,8,14] Therefore, we detected the alterations in the expressions of TNFα, IL-6, IL-1β, and IL-17A after IL-17C neutralization post-IR. As shown in Fig. 2a–d, renal ischemia/reperfusion led to significantly increased levels of TNFα, IL-6, and IL-1β, which were reduced by post-IR administration of IL-17C antibody. Consistent with results of mRNA expression, flow cytometry experiments indicated that IRI induced Th17 cells activation was significantly inhibited by IL-17C neutralization (Fig. S2a, Fig. 2e). We also detected the alteration of $CD4^+FOXP3^+$ Treg cells proportion in renal tissue and our data demonstrated that there was no significant difference in Treg cell differentiation in AKI mice (Fig. S2a and S3a and b). This indicates that IL-17C had no effects on Treg cell differentiation in the current experiments. Since pro-inflammatory chemokines can recruit various inflammatory cells such as neutrophils and monocytes/macrophages, we further determined whether IL-17C blockade could affect renal inflammatory cell infiltration. Our results suggested that renal MPO^+ neutrophils and $F4/80^+$ macrophages were significantly increased in IRI mice. This recruitment was blunted by IL-17C neutralizing antibody (Fig. 2f and g).

IL-17C was up-regulated via NF-κB pathway in kidneys of IRI mice *in vivo* and hypoxia-treated HK2 cells *in vitro*

We next explored the signaling pathways involved in the regulation of IL-17C expression in AKI mouse model. As NF-κB was reported as a critical transcription factor for IL-17C, we conducted a series of experiments to investigate the possible mechanisms.[6] As shown in Fig. 3a, NF-κB activity increased significantly in IRI mice, whereas IL-17C neutralization significantly abrogated activation of NF-κB, indicating that IL-17C was a potential activator for NF-κB. We further investigated whether NF-KB activation was involved in the induction of IL-17C expression using a siRNA targeting NF-κB p65. As illustrated in Fig. 3b and c, NF-κB p65 mRNA expression was successfully reduced to 50% of the basal level following siRNA treatment, and down-regulation of p65 expression in mice after renal ischemia was accompanied by a remarkable

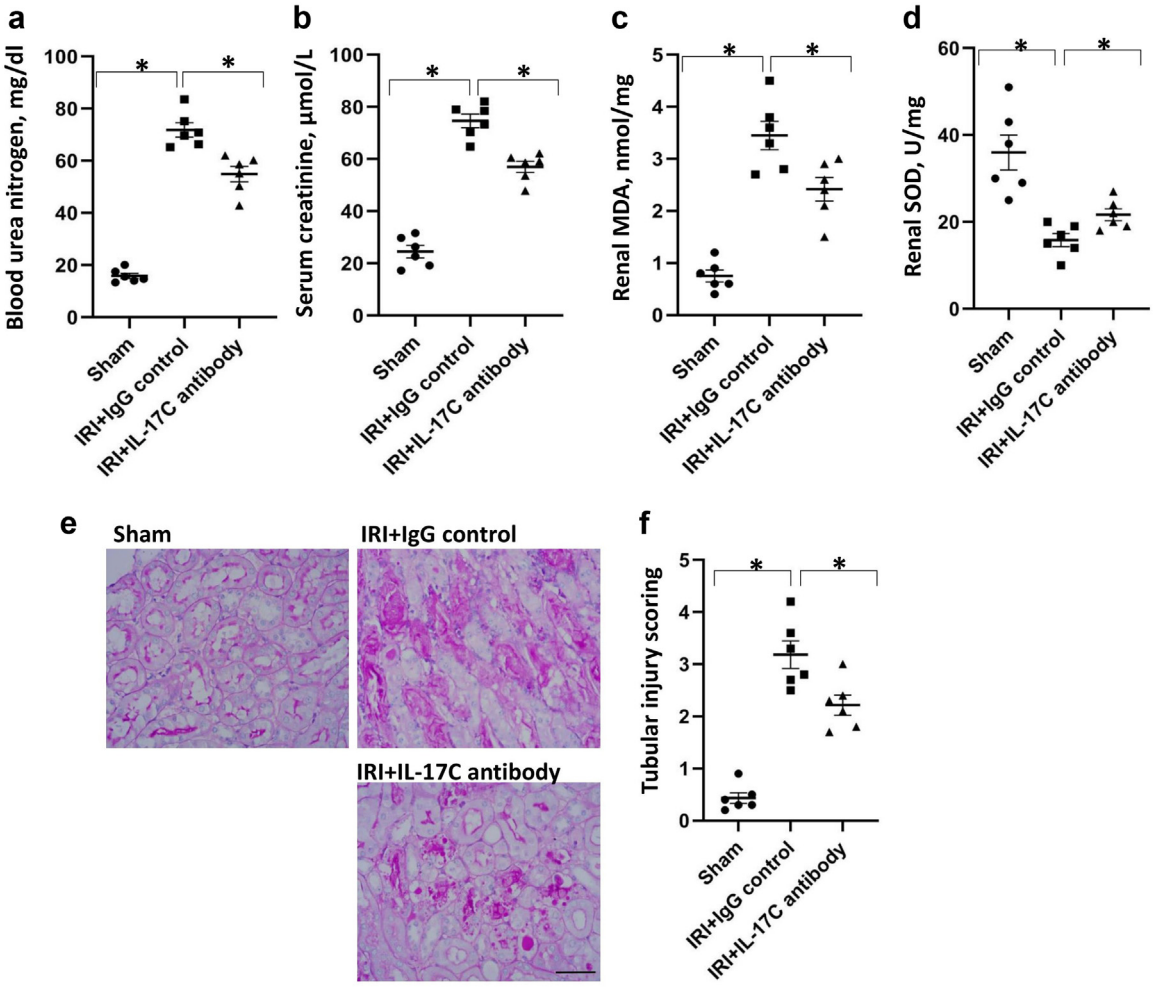

Fig. 1: **Post-IR blockade of IL-17C attenuated AKI in mice.** 12-week-old male C57BL/6JGpt mice were conducted by bilateral renal ischemia for 30 min and tissue collection was conducted 24 h after reperfusion. IL-17C antibody or normal control IgG (10 mg/kg) were administered 3 h after reperfusion via tail vein injection. Kidney sections were stained with periodic acid–Schiff to evaluate renal histologic injury. (**a**) Blood urine nitrogen. (**b**) Serum creatinine. (**c**) Renal MDA level. (**d**) Renal SOD level. (**e**) Representative images of renal sections with PAS staining (original magnification, 200×, bar = 50 μm). (**f**) Quantification of tubular injury scores. All values are presented as mean ± SEM (n = 6 for each group, *p < 0.05, one-way ANOVA followed by Holm–Šidák post hoc test).

decrease of IL-17C mRNA expression. Since IL-17C is preferentially produced by epithelial cells, human renal epithelial cells (HK2) were utilized to further investigate whether NF-κB mediated IL-17C up-regulation upon hypoxia stimulation. The results showed that knockdown of p65 abolished hypoxia-induced IL-17C up-regulation in HK2 cells (Fig. 3d–f). Additionally, we investigated whether HIF-1α pathway was involved in the induction of IL-17C expression. 3-(5-hydroxymethyl-2-furyl)-1-benzylindazole (YC-1) was used to inhibit HIF-1α in IRI mice. Our data demonstrated that IL-17C mRNA expression was not affected by HIF-1α inhibition *in vivo* (Fig. S4a). For *in vitro* experiments, HIF-1α Decoy were used to block HIF-1α, while $CoCl_2$ was administrated to stabilize HIF-1α expression in HK2 cells. Renalase, one of the target genes of HIF-1α, was utilized as a positive control to verify the blockade efficiency of HIF-1α Decoy. As illustrated in Fig. S4b and c, $CoCl_2$ and hypoxia both induced renalase up-regulation, while hypoxia induced renalase up-regulation was significantly abrogated by HIF-1α Decoy. In contrast, chemical induction of HIF-1α by $CoCl_2$ could not up-regulate IL-17C, whereas hypoxia significantly induced up-regulation of IL-17C in HK2 cells (Fig. S4d), indicating that HIF-1α itself could not promote IL-17C activation. Moreover, we found that hypoxia induced IL-17C up-regulation was not affected by HIF-1α Decoy, suggesting that HIF-1α might not be involved in the hypoxia induced IL-17C up-regulation. Taken together, these data suggested

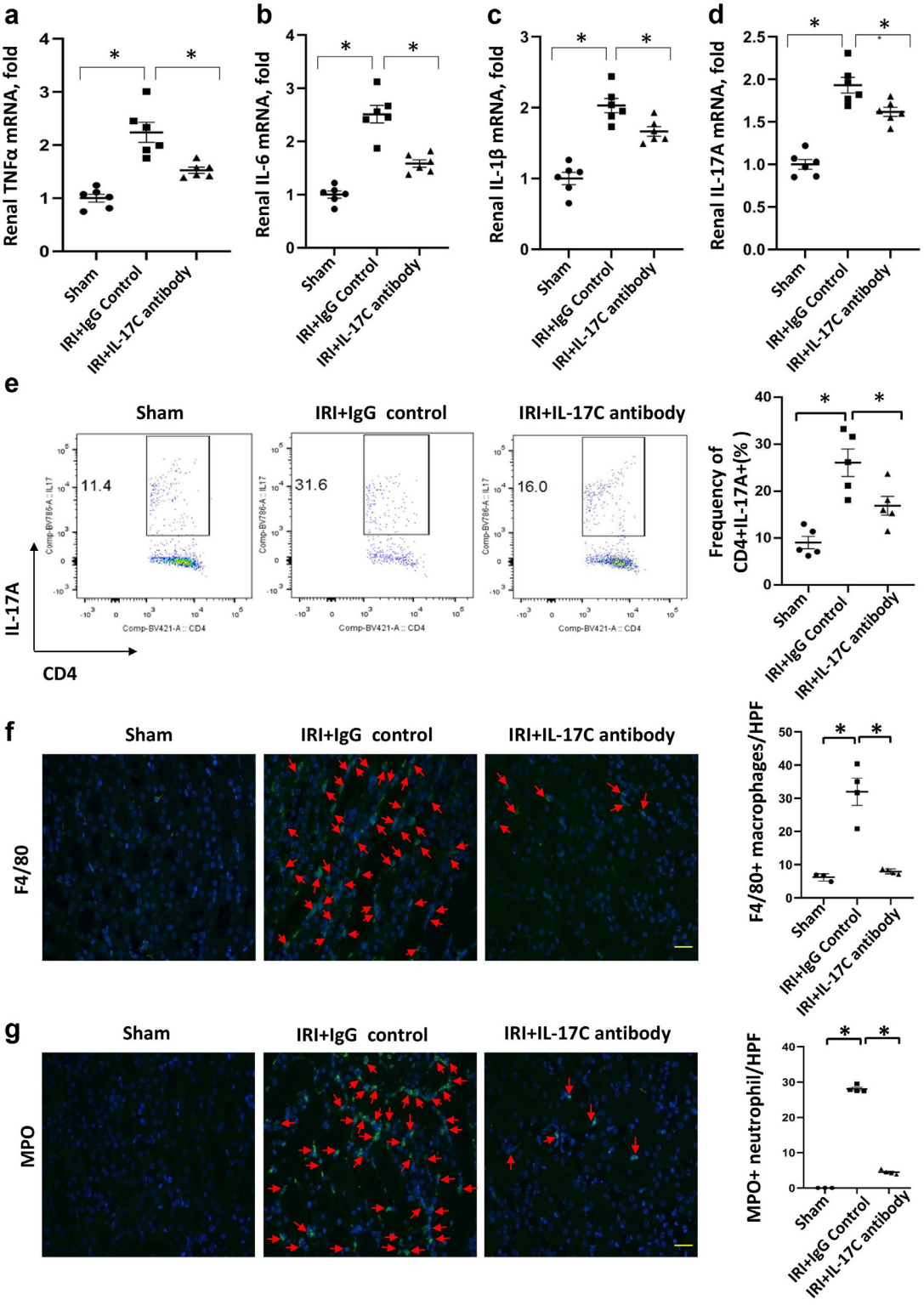

Fig. 2: **Delayed IL-17C neutralization reduced renal inflammation and Th17/IL-17A activation in IRI mice.** C57BL/6JGpt mice were given IL-17C neutralizing antibody or normal control IgG (10 mg/kg) 3 h after reperfusion via tail vein injection, and tissue collection was conducted 24 h after reperfusion. TNFα, IL-6, IL-1β and IL-17A, mRNA expression in mouse kidney were assessed. (**a**) Renal TNFα mRNA expression. (**b**) Renal

that NF-κB instead of HIF-1 pathway activation was essential to IL-17C induction under IRI conditions *in vivo* and hypoxia *in vitro*.

Renal IL-17C was up-regulated in diabetic nephropathy

To explore whether IL-17C was associated with the development of DN, we examined the expression of renal IL-17C in kidney biopsy specimens from patients with DN and in kidneys from a *db/db* mice model. Our results showed that renal IL-17C mRNA levels were significantly up-regulated in kidney biopsy specimens from patients with DN compared with those from control subjects (Fig. 4a). As shown in Table 2, there was no difference in age, gender, blood pressure between DN and control subjects. We also exclude infection in the DN subjects. There was no significant difference between two groups in the levels of IL-17RE (Fig. 4b). Furthermore, the levels of IL-6 and IL-1β were remarkably higher in the patients with DN compared with those in control subjects (Fig. 4c and d). Additionally, results of immunohistochemistry staining and *in situ* hybridization (Fig. 4e) indicated that IL-17C was significantly up-regulated in kidney biopsy specimens from patients with DN compared with those from control subjects. Notably, IL-17C was primarily expressed in renal tubular epithelial cells rather than interstitial cells or glomerular cells. Consistent with the data in human, the expression of renal IL-17C, IL-6, and IL-1β was increased in db/db mice compared to that in db/mice. However, there was no significant difference in the expression of IL-17RE (Fig. 4f–j).

IL-17C blockade attenuated the development of nephropathy in *db/db* mice

To further determine whether IL-17C signaling pathway was involved in the pathogenesis of DN, an IL-17C neutralizing antibody was used in *db/db* mice and renal injury was evaluated. As demonstrated in Fig. 5a–g, IL-17C blockade did not affect the blood glucose, insulin, HbA1C, body weight, and serum creatinine levels in *db/db* mice. However, IL-17C neutralization effectively mitigated renal injury in *db/db* mice evidenced by decreased albuminuria and ratios of kidney weight to body weight (kidney weight index) compared with control antibody treatment. Moreover, pathological analysis showed that IL-17C neutralizing antibody-treated *db/db* mice exhibited decreased mesangial matrix accumulation, and restored podocytes density compared with un-treated *db/db* mice, as shown in Fig. 5h and i. These data indicated that IL-17C neutralization might prevent renal injury through inhibition of mesangial expansion, collagen deposition, and podocytes loss.

Expression of pro-inflammatory cytokines, Th17 cells activation, and renal inflammatory cells infiltration were blunted by IL-17C neutralization in *db/db* mice

In line with those results (Figs. 2e and 4a–d) that IL-17C blockade diminished expression of pro-inflammatory cytokines and Th17 cells activation in AKI mice, IL-17C neutralization also attenuated the up-regulation of renal monocyte chemotactic protein-1 (MCP-1), IL-6, and IL-1β expression in *db/db* mice (Fig. 6a–c). Furthermore, IL-17A, one of IL-17 cytokine family, was significantly up-regulated in *db/db* mice compared with control subjects, which was remarkably abrogated by IL-17C neutralization (Fig. 6d). Consistent with results of mRNA expression, diabetes induced Th17 cells activation was significantly inhibited by IL-17C neutralization (Fig S2b, Fig. 6e). Consistently, there was no difference in Treg cells differentiation in *db/db* mice (Fig. S2b and S4c and d). In addition, immunofluorescence of F4/80 revealed that the tubulointerstitial infiltration of $F4/80^+$ macrophages were significantly increased in *db/db* mice, and were blunted by IL-17C neutralizing antibody administration (Fig. 6f).

IL-17C knockdown attenuated high glucose-induced inflammation *in vitro*

Our data indicated that hyperglycemia induced up-regulation of pro-inflammatory cytokines such as IL-6 and IL-1β in HK2 cells. IL-17C siRNA was used to examine whether IL-17C was involved in hyperglycemia induced inflammation. As expected, IL-17C knockdown using siRNA remarkably decreased the elevation of IL-6 and IL-1β mRNA expression in HK2 cells in exposure to high glucose (Fig. 7a–c). Consistently, ELISA analysis of IL-6 and IL-1β levels in culture supernatants of HK2 cells revealed that IL-17C knockdown decreased high glucose-induced secretion of IL-6 and IL-1β in HK2 cells (Fig. 7d and e).

IL-17C was up-regulated via NF-κB in kidneys of *db/db* mice *in vivo* and high glucose treated HK2 cells *in vitro*

We next explored the signaling pathways involved in the regulation of IL-17C expression in *db/db* mice. As

IL-6 mRNA expression. (**c**) Renal IL-1β mRNA expression. (**d**) Renal IL-17A mRNA expression. (**e**) Representative fluorescence-activated cell sorting (FACS) images and quantitatively analysis of $CD4^+IL\text{-}17A^+$ Th17 cells in IRI mice. (**f**) Representative images of immunofluorescence against macrophage marker F4/80 and numbers of $F4/80^+$ macrophages per high-power field (HPF) in IRI mice. (**g**) Representative images of immunofluorescence against neutrophils marker MPO and numbers of MPO^+ neutrophils per HPF in AKI mice. Original magnification, 400×, bar = 50 μm. All values are presented as mean ± SEM (n = 5∼6 for each group, *p < 0.05, one-way ANOVA followed by Holm–Šidák post hoc test).

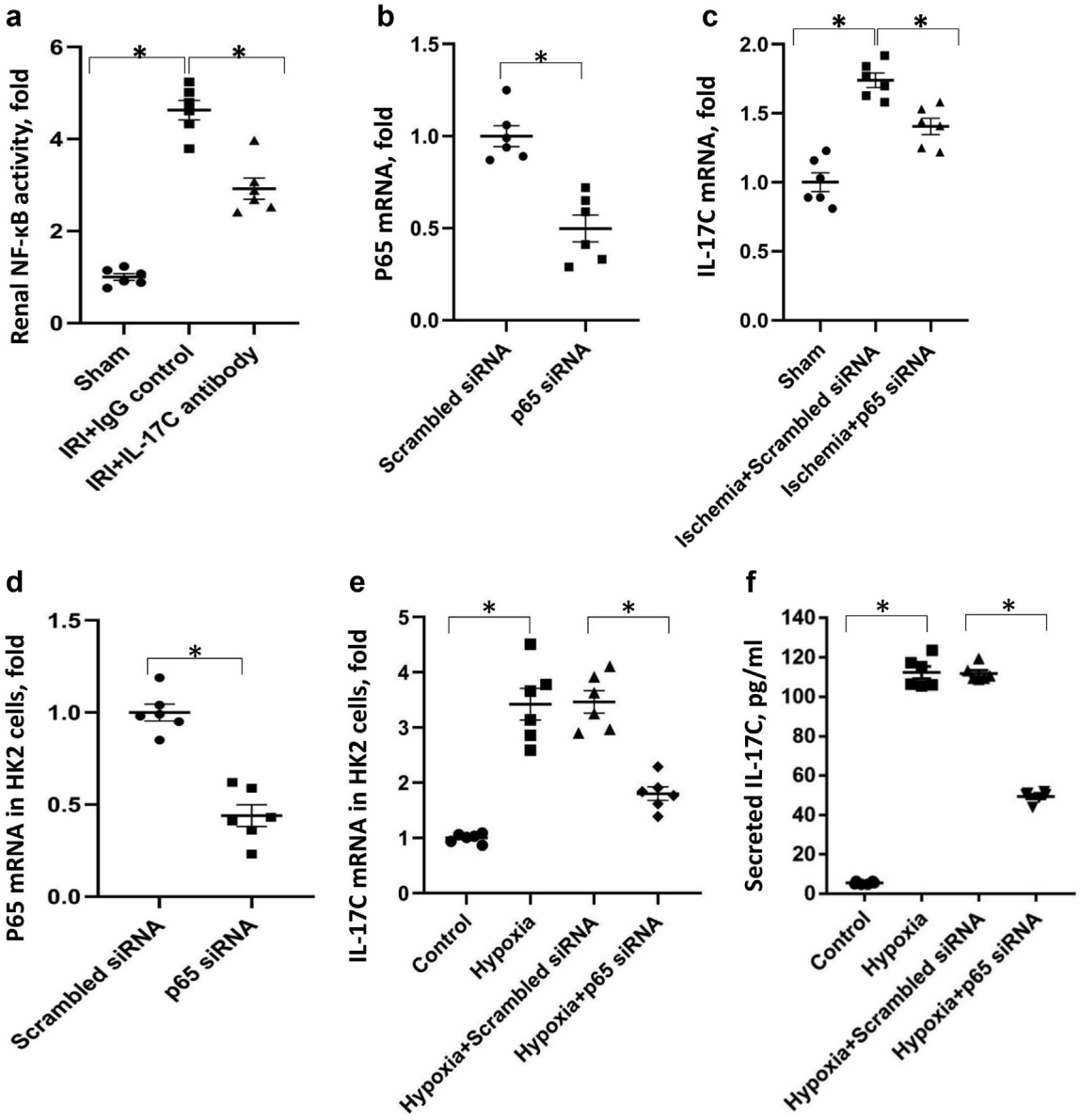

***Fig. 3:* IL-17C was up-regulated via NF-κB in kidneys of IRI mice *in vivo* and hypoxia treated HK2 cells *in vitro*.** C57BL/6JGpt mice were given IL-17C antibody or normal control IgG (10 mg/kg) 3 h after reperfusion via tail vein injection. (**a**) Renal NF-κB activity in mouse model of IRI. (**b**) Renal p65 mRNA expression with p65 siRNA. (**c**) Renal IL-17C mRNA expression after ischemia with p65 siRNA. Scrambled siRNA or p65 siRNA (10 nmol/d) was injected intraperitoneally for three successive days before ischemia. All the mice were sacrificed 30 min after ischemia and kidney tissues were harvested. HK2 cells were pre-treated with p65 siRNA were pre-treated with p65 siRNA before hypoxia (1% O_2) induction for 6 h. (**d**) p65 mRNA expression. (**e**) IL-17C mRNA expression after hypoxia induction in HK2 cell treated with p65 siRNA. mRNA levels are expressed as x-fold of controls. (**f**) IL-17C levels in culture supernatants of HK2 cells exposed to hypoxia for 24 h with or without p65 siRNA treatment. All values are presented as mean ± SEM (n = 6 for each group, *p < 0.05, calculated using one-way ANOVA followed by Holm–Šidák post hoc test for all panels, except for **b** and **d**, where 2-tailed t test was used).

shown in Fig. 8a, NF-κB activity increased significantly in *db/db* mice, whereas IL-17C neutralization significantly abrogated activation of NF-κB, indicating that IL-17C was a potential activator for NF-κB. We further investigated whether NF-κB activation was involved in the induction of IL-17C expression using a siRNA targeting NF-κB p65. As illustrated in Fig. 8b and c, knockdown of NF-κB p65 significantly decreased IL-17C mRNA expression in *db/db* mice. In addition, our data showed that IL-17C was significantly increased upon

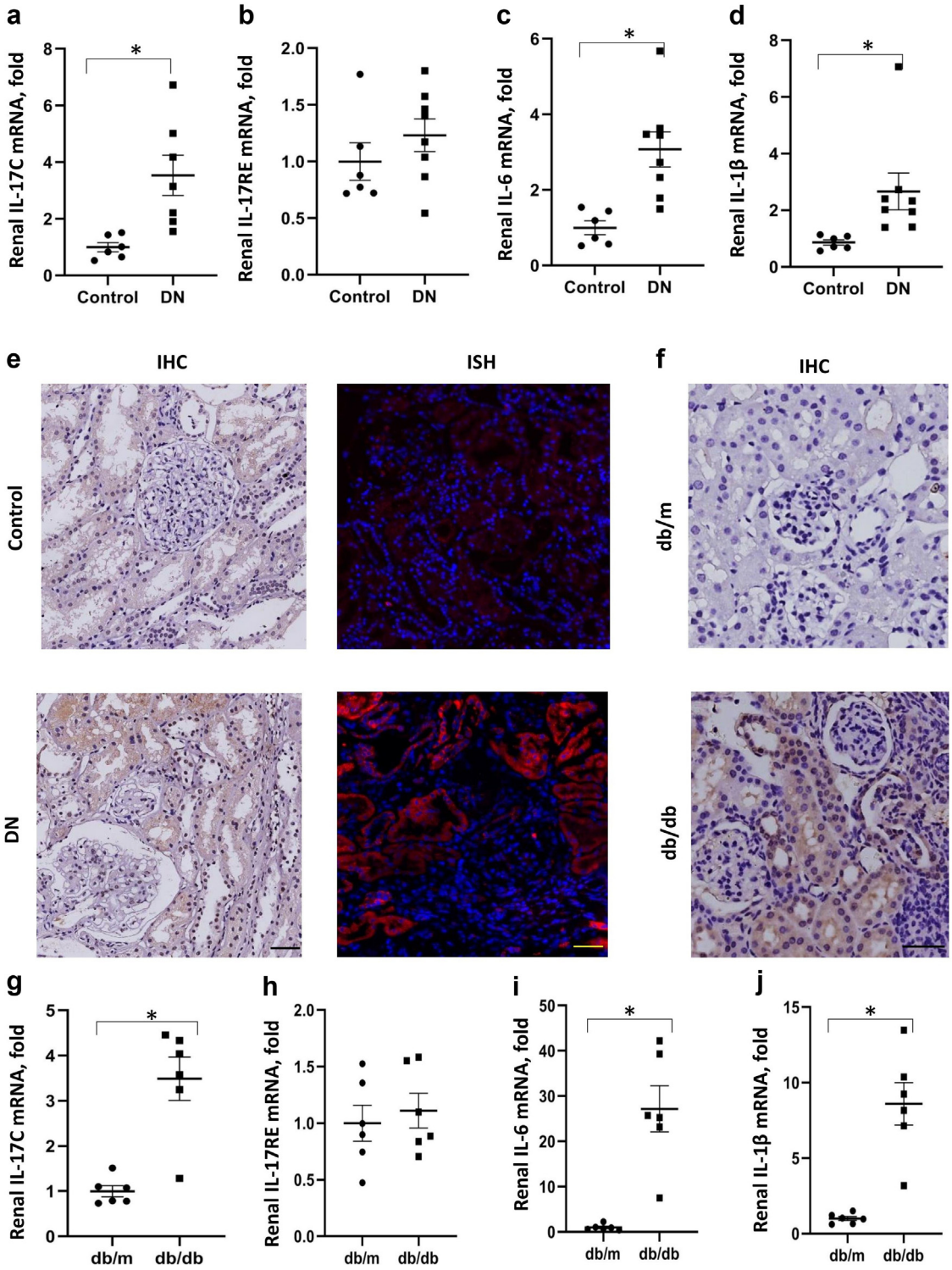

Fig. 4: **Renal IL-17C was up-regulated in diabetic nephropathy.** Renal expression of IL-17C, IL-17RE, IL-6, and IL-1β were examined using quantitative reverse-transcription polymerase chain reaction (**a–e**) or mouse kidneys with diabetic nephropathy (**f–j**). (**a**) Renal IL-17C mRNA expression. (**b**) Renal IL-17RE mRNA expression. (**c**) Renal IL-6 mRNA expression. (**d**) Renal IL-1β mRNA expression in human kidney biopsy. (**e**) Representative image of IL-17C expression in kidney biopsy specimens from patients with DN. Left panel: immunohistochemistry (IHC, original magnification, 400×, bar = 50 μm). Right panel: *in situ* hybridization (ISH, original magnification, 200×, bar = 50 μm). (**f**) Representative IHC

hyperglycemia stimulation in HK2 cells, and knockdown of p65 significantly decreased high glucose-induced up-regulation of IL-17C expression in HK2 cells as shown in Fig. 8d and e.

Discussion

In the current study, we demonstrated that renal IL-17C was up-regulated in human beings and mice with DN. We also found that IL-17C neutralization alleviated both IRI-induced AKI and diabetes-associated chronic kidney injury through the mechanisms associated with inhibition of pro-inflammatory cytokine production, inflammatory cells infiltration, Th17/IL-17A axis activation, and NF-κB pathways. Our results indicated that hypoxia or high glucose-induced IL-17C expression *in vivo* or *in vitro* was regulated by NF-κB pathways instead of HIF-1α pathways. Therefore, we suggest that IL-17C activation via NF-κB under AKI and diabetes conditions contributes to the pathogenesis of AKI and DN.

Accumulated evidence has proven that IL-17C participates in the development of renal disease. Recently, Sonja et al. identified serum IL-17C as the only IL-17 family cytokine with elevated serum protein levels in patients with ANCA-associated crescentic glomerular nephropathy.[5] Our previous studies further demonstrated a marked elevation of renal IL-17C in human and mice kidneys with AKI and confirmed the therapeutic effects of IL-17C blockade in AKI.[14] This study found that up-regulation of IL-17C was also present in patients with DN and *db/db* mice, suggesting that IL-17C might play a crucial role in the pathogenesis of chronic inflammation associated renal diseases such as DN.

The inflammatory response in renal disease involves an orchestrated interplay between the tubular epithelium and immune system, that initiates and drives a pathogenic inflammatory response. In the present study, we proved that hypoxia and hyperglycemia could induced IL-17C up-regulation and elevated secretion of pro-inflammatory cytokines. Since IL-17C is reported to be primarily induced in renal tubular cells, we speculated that IL-17C exerts its biological effects by amplifying inflammatory responses in two alternative ways: 1) IL-17C leads to activation and recruitment of various types of inflammatory cells such as Th17, macrophage, and neutrophil; 2) IL-17C induces resident cells, such as tubular cell, fibroblast, and macrophage to secrete pro-inflammatory cytokines in an autocrine or paracrine manner.[3,8,21,22] IL-17C could also induce the expression of pro-inflammatory cytokines and chemokines not only in immune cells but also in epithelial cells, which primarily express IL-17RE.[2,3,8,14,23] Previous studies have demonstrated that IL-17C and IL-17RE deficient mice exhibited attenuated crescentic glomerulonephritis, which was accompanied by reduced Th17 cell activation, IL-17A expression and neutrophils infiltration.[5] In agreement with our previous study, we found that Th17/IL-17A axis was significantly activated in both AKI and *db/db* mice, while inhibition of IL-17C remarkably reduced the expression of IL-17A and Th17 cell infiltration. However, IL-17C neutralization had no effects on the Treg cell infiltration in both models. Consistent with results of Th17 cells, IL-17C blockade led to blunted macrophage infiltration both in AKI and DN mice. Apart from Th17 cell and macrophage recruitment, neutrophil recruitment has also been identified as an important downstream effect of IL-17C/Th17 cell activation. We found distinct results to IL-17C/Th17 activation in AKI and DN models. In AKI mice, the acute inflammatory model, MPO^+ neutrophils were remarkably increased in response to IL-17C activation, while inhibition of IL-17C significantly reduced neutrophils infiltration. In contrast, there was no significant change in MPO^+ neutrophils in *db/db* mice with or without IL-17C blockade (data not shown). These results indicated that IL-17C induced neutrophil recruitment might be only involved in the pathogenesis of acute injury rather than chronic inflammation associated renal disease. On the other hand, our data also

	Control	Diabetic nephropathy
N	6	8
Age (year)	43.0 ± 6.8	53.0 ± 3.4
Gender	2 M, 4 F	7 M, 1 F
Race	Asian	Asian
Pathological findings	Minimal change or no obvious renal injury	Diabetic nephropathy
FPG (mmol/L)	4.7 ± 0.2	7.6 ± 0.5[a]
HbA1c, %	5.1 ± 0.1	7.2 ± 0.5[a]
Alanine transaminase [ALT] (U/L)	23.9 ± 7.8	15.3 ± 1.7
Aspartate transaminase [AST] (U/L)	36.7 ± 13.3	18.7 ± 1.5
BUN (mmol/L)	6.1 ± 0.3	9.5 ± 1.8
Scr (μmol/L)	84.0 ± 12.0	115.8 ± 11.3
Urine protein, g/24 h	0.1 ± 0.0	3.9 ± 1.0[a]
Systolic blood pressure [SBP] (mmHg)	128 ± 10	144 ± 12
Diastolic blood pressure [DBP] (mmHg)	83 ± 6	84 ± 4
White blood cell [WBC] ($\times 10^9$/L)	7.2 ± 0.8	7.1 ± 0.5
Urine culture test	Negative	Negative
Urinary WBC (mm^3)	21.2 ± 11.4	15.5 ± 6.2

[a]$p < 0.05$ vs control.

Table 2: **Clinical characteristics of the patients with diabetic nephropathy.**

image of IL-17C expression in kidney biopsy specimens from IRI mice (original magnification, 400×, bar = 50 μm). (**g**) Renal IL-17C mRNA expression. (**h**) Renal IL-17RE mRNA expression. (**i**) Renal IL-6 mRNA expression. (**j**) Renal IL-1β mRNA expression in *db/db* mice. mRNA levels are expressed as x-fold of controls. All values are presented as mean ± SEM (**a–e**) n = 6 for control subjects, n = 8 for patients with diabetic nephropathy. (**f–j**) n = 6 for each group (*p < 0.05, 2-tailed t test).

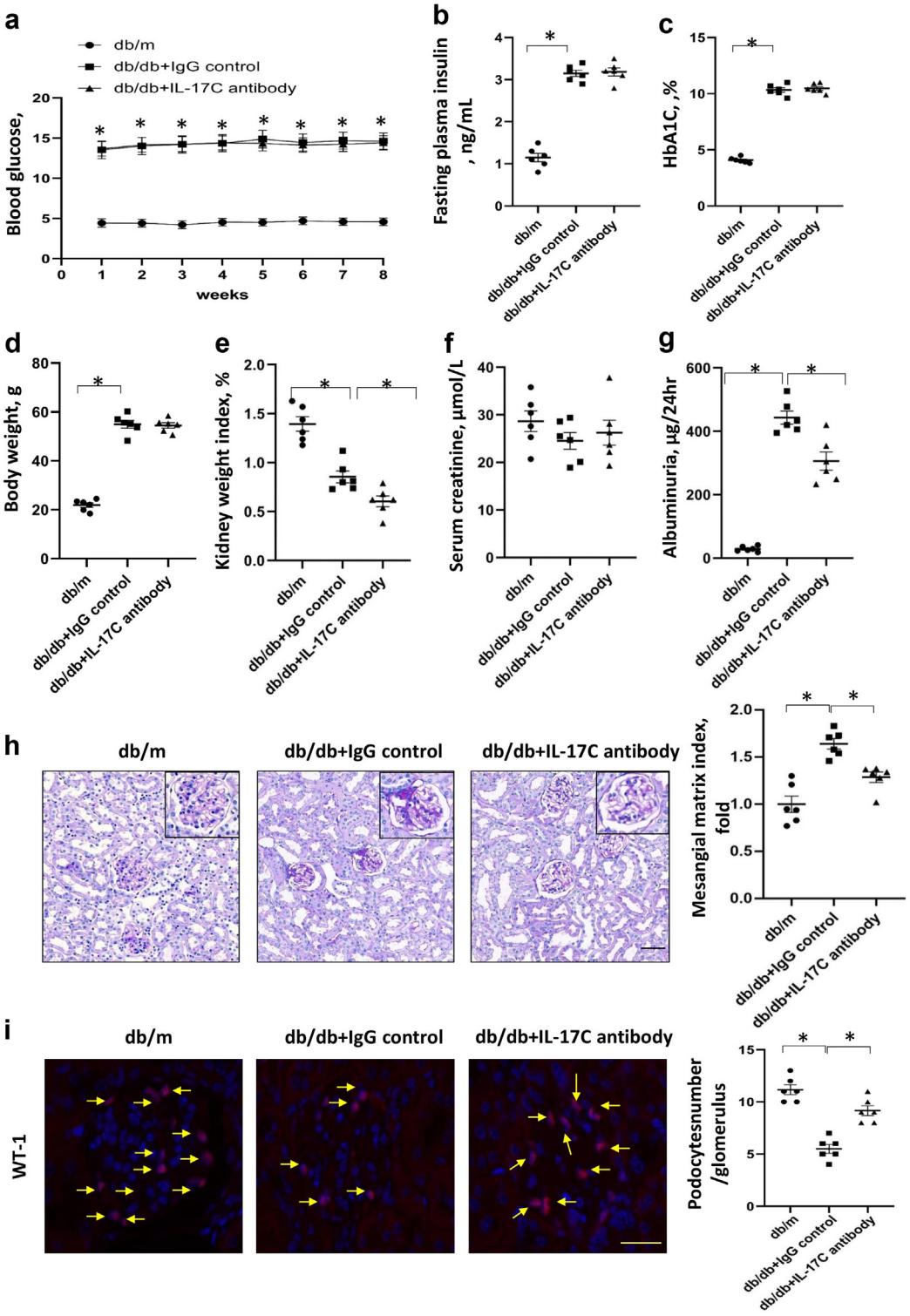

Fig. 5: **IL-17C neutralization attenuated kidney injury in *db/db* mice.** 12-week-old male *db/db* mice were treated with IL-17C neutralizing antibody or normal control IgG (10 mg/kg, twice a week) via tail vein injection and tissue collection was performed after 8 weeks. (**a**) Fasting blood glucose levels were assessed at different time points. (**b**) Fasting plasma insulin. (**c**) HbA1C. (**d**) Body weight. (**e**) Kidney weight index. (**f**) Serum creatinine. (**g**) Albuminuria for 24 h. (**h**) Representative images of renal sections with PAS staining (top panel, original magnification, 200×,

demonstrated that up-regulated renal IL-17C was accompanied by increased expression of TNFα, IL-6, and IL-1β, whereas inhibition of IL-17C significantly reduced the expression of these pro-inflammatory cytokines. These results indicated that IL-17C might function as an important cytokine for initiating and amplifying acute or chronic inflammation in inflammatory associated kidney diseases. For instance, initial insult may induce IL-17C up-regulation in resident cells, which activate non-injured kidney cells or immune cells to amplify inflammatory response and ultimately led to tissue damage. IL-17C might amplify inflammatory response via Th17/IL-17A activation, neutrophil recruitment, macrophage infiltration as well as induction of pro-inflammatory cytokines and chemokines.

In the present study, different patterns of immune cell infiltration in renal histological structures were found in AKI and DN models. This is not surprising because these two models are distinct inflammation-associated renal diseases (acute versus chronic). Aforementioned data indicated that neutrophil recruitment was only affected in AKI with or without IL-17C blockade. Basolateral release of IL-17C by tubular cells can interact with infiltrating immune cells, thereby amplifying inflammation and mediating tissue injury in the renal IRI model. Nevertheless, the underlying mechanism by which IL-17C promotes chronic glomerular damage in *db/db* mice remains elusive. Our data demonstrated that interstitial macrophage infiltration was diminished by IL-17C blockade, indicating that IL-17C mediated tissue injury, at least in part, via the recruitment and activation of macrophage. It has been shown that IL-17C can induce expression of IL-17RE in murine mesangial cells.[5] Consistently, we found that IL-17C neutralization attenuated mesangial expansion in *db/db* mice. Thus, we speculate that IL-17C secreted by renal tubular epithelial cells may bind to IL-17RE in mesangial cells to mediate mesangial expansion and cause diabetic glomerular damage. Additionally, we found that activation of Th17/IL-17A axis was also blunted by IL-17C inhibition. Previous studies have proved that IL-17C promotes Th17 cell responses and immune-mediated glomerular diseases via IL-17RE expressed on $CD4^+$ Th17 cells and IL-17C-driven tissue injury in crescentic glomerulonephritis is IL-17A-dependent.[5] In line with our data, glomerular macrophages were diminished in IL-17RE knockout mice. These results indicate that Th17 activation may be mainly responsible for glomerular inflammation and macrophage infiltration. Crescentic glomerulonephritis, however, is characterized by presence of extensive glomerular crescents as the principal histologic finding, which may be different from DN. So far, we have not been able to confirm the precise mechanism of how IL-17C promotes inflammation in diabetic glomeruli, which need further studies in future.

Given that IL-17C is a target gene of NF-κB, we speculated hyperglycemia and hypoxia-induced NF-κB activation might account for IL-17C up-regulation in AKI and DN. This hypothesis was supported by our data. Knockdown of NF-κB p65 significantly reduced IL-17C expression *in vivo*, *in vitro* as well as under hypoxia and hyperglycemic conditions. Through our investigation of HIF-1α, we found that blocking HIF-1α or stabilizing HIF-1α expression had no effects on IL-17C expression in response to hypoxia and high glucose stimulation. We also found that blockade of IL-17C could inhibit NF-κB activation in diabetic and IRI mice. Consistent with our results, intestinal IL-17C promotes the synthesis and release of inflammatory factors by activating the NF-κB pathways.[3] In summary, these data indicated that renal IL-17C up-regulation induced by hyperglycemia and hypoxia was NF-κB dependent, and in turn, IL-17C might further activate NF-κB, and these synergistic effects collectively led to inflammation amplification, which might participate in the pathogenesis of AKI and DN.

Although hypoxia and high glucose are two different stimuli, they could induce similar damage processes to tubular epithelial cells.[14,24,25] It has been suggested that injured cells can release damage-associated molecular patterns (DAMPs), which can bind to the innate immune receptors, thereby triggering the inflammatory response.[26] Recently, mounting evidence has suggested that DAMPs are essentially responsible for sensing endogenous stimulations and activating the inflammatory response in acute and chronic sterile inflammation. Previous reports have proved that DAMPs released in renal ischemia/reperfusion injury causes the activation of the IL-23/IL-17A axis. However, understanding whether DAMPs can induce IL-17C activation requires further study.

It is generally reported that other IL-17 family of cytokines also play an important role in the pathogenesis of renal disease.[2,27] IL-17A is a best-studied member of IL-17 family cytokines and has been widely identified as an important regulator in several kidney diseases, including AKI and DN.[13,28–31] There is now consensus that IL-17A contribute the pathogenesis of AKI.[28,31] However, there was controversy in the role of IL-17A

bar = 50 μm. The upper right corner is an enlarged part of the glomerulus) and semi-quantitative analysis of mesangial matrix. (**i**) Representative immunofluorescence staining of WT-1 (bottom panel, 400×, bar = 25 μm) and the average number of podocytes observed of a total of 25 glomeruli per animal. All values are presented as mean ± SEM (n = 6 for each group, *p < 0.05, one-way ANOVA followed by Holm–Šidák post hoc test).

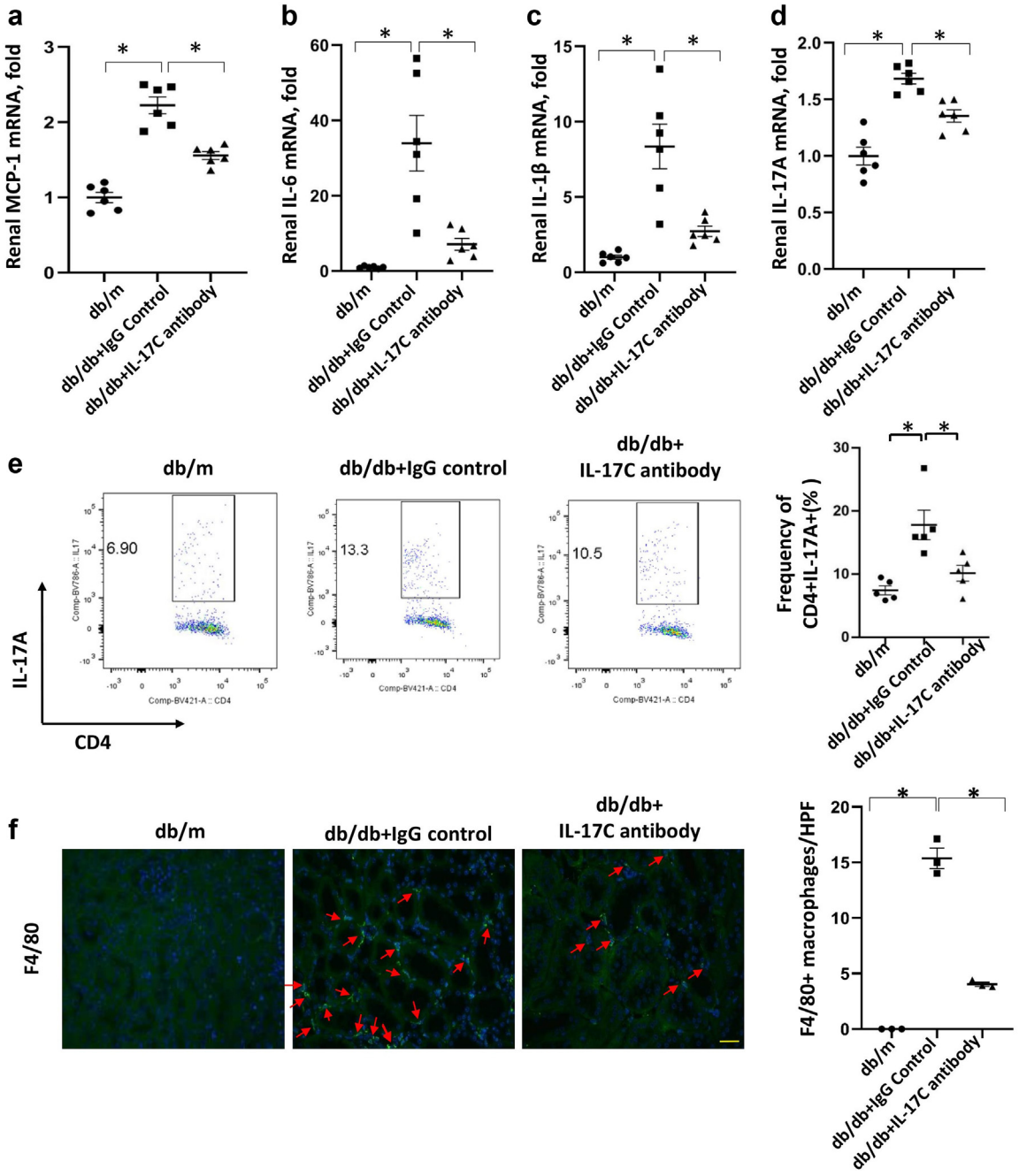

Fig. 6: **IL-17C neutralization reduced renal inflammation and Th17/IL-17A response in *db/db* mice.** 12-week-old male *db/db* mice were treated with IL-17C neutralizing antibody or normal control IgG (10 mg/kg, twice a week) via tail vein injection and tissue collection was performed after 8 weeks. (**a**) Renal MCP-1 mRNA expression. (**b**) Renal IL-6 mRNA expression. (**c**) Renal IL-1β mRNA expression. (**d**) Renal IL-17A mRNA expression. (**e**) Representative FACS images and quantitatively analysis of $CD4^{+}IL\text{-}17A^{+}$ Th17 cells in *db/db* mice. (**f**) Representative images of immunofluorescence staining of F4/80 immunofluorescence against macrophage marker F4/80 and numbers of $F4/80^{+}$ macrophages per HPF in *db/db* mice (original magnification, 400×, bar = 50 μm). All values are presented as mean ± SEM (n = 6 for each group, *p < 0.05, one-way ANOVA followed by Holm–Šidák post hoc test).

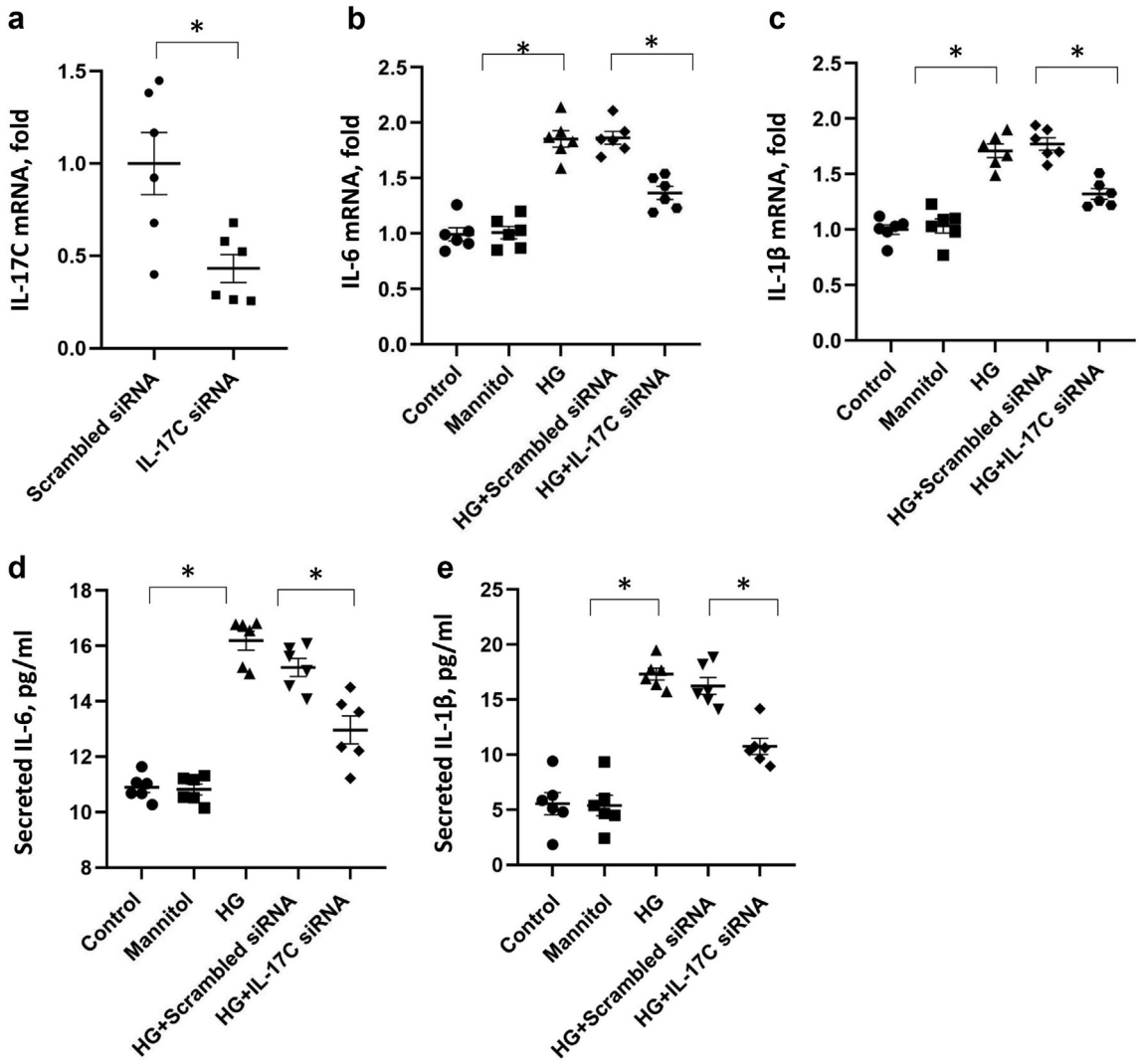

***Fig. 7*: IL-17C knockdown attenuated high glucose-induced inflammation *in vitro*.** (**a**) Knockdown efficiency of IL-17C. IL-6 (**b**), and IL-1β (**c**) mRNA expression in HK2 cell exposed to high glucose for 24 h with or without IL-17C siRNA treatment. mRNA levels are expressed as x-fold of controls. ELISA analysis of IL-6 (**d**) and IL-1β (**e**) levels in culture supernatants of HK2 cells exposed to high glucose for 24 h with or without IL-17C siRNA treatment. All values are presented as means ± SEM (n = 4∼6 for each group, *p < 0.05, calculated using one-way ANOVA followed by Holm–Šidák post hoc test for all panels, except for **a**, where 2-tailed t test was used).

in DN. Mohamed et al. found that low dose of IL-17A administration effectively prevented renal injury in both STZ-induced and *db/db* diabetic mice; Meanwhile, protective effects were also observed after administration of IL-17F but not IL-17C or IL-17E.[32] In contrast, other study found that IL-17A blockade ameliorated renal dysfunction and disease progression in BTBR ob/ob mice.[20] Recent study showed that IL-17E could regulate macrophage phenotype in kidney and reduce renal ischemic/reperfusion injury.[33] Thus, other IL-17 family of cytokines may participate in the pathogenesis of AKI and DN. In this study, we did not control for the effects of other IL-17 family cytokines. This is a limitation of the present study.

There were several other limitations in this study. First, in addition to the NF-κB pathway, other signaling pathways may participate in hypoxia or high glucose mediated IL-17C induction. This is an area that requires further study. Second, further mechanistic studies are needed to fully examine underlying downstream mechanisms of IL-17C-elicited inflammation in diabetic glomeruli. For instance, the effects of IL-17RE knockdown on experimental DN were not investigated in this study. Additionally, the number of renal biopsy

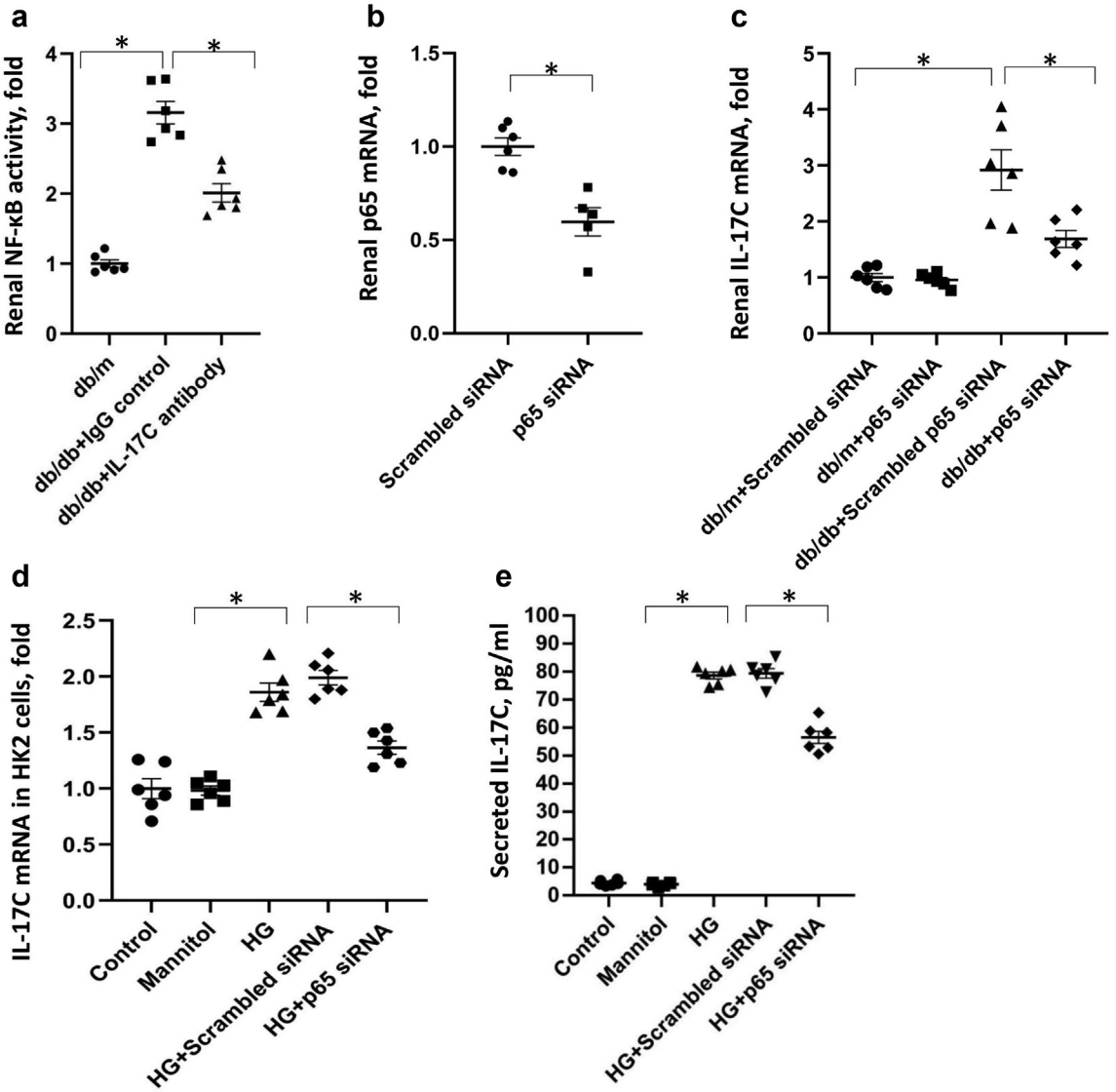

Fig. 8: **IL-17C was up-regulated via NF-κB in kidneys of *db/db* mice *in vivo* and high glucose treated HK2 cells *in vitro*.** 12-week-old male *db/db* mice treated with IL-17C antibody or normal control IgG (10 mg/kg, twice a week) via tail vein injection and tissue collection was performed after 8 weeks. (**a**) Renal NF-κB activity in *db/db* mice. (**b**) Renal p65 mRNA expression with p65 siRNA. (**c**) Renal IL-17C mRNA expression in *db/db* mice with p65 siRNA. Scrambled siRNA or p65 siRNA (10 nmol/d) was injected intraperitoneally for three successive days in *db/db* mice at 20 weeks of age before mice were sacrificed for tissues collection. HK2 cells were cultured in the presence of either normal glucose (NG) or high glucose (HG) for 24 h after pre-treatment of p65 siRNA or IL-17C siRNA. (**d**) IL-17C mRNA expression after high glucose treatment in HK2 cell treated with p65 siRNA. mRNA levels are expressed as x-fold of controls. (**e**) IL-17C levels in culture supernatants of HK2 cells exposed to hyperglycemia for 24 h with or without p65 siRNA treatment. All values are presented as means ± SEM (n = 4∼6 for each group, *p < 0.05, calculated using one-way ANOVA followed by Holm–Šidák post hoc test for all panels, except for **b**, where 2-tailed t test was used).

specimens for IL-17C expression was limited and the subjects were randomly enrolled and not age or gender matched to avoid the confounding effects. Therefore, the relationship between IL-17C expression and DN progression should be confirmed in a larger population of patients in the future.

In conclusion, this study identified a pathogenic role of IL-17C in DN and AKI. It was deduced that renal IL-17C up-regulation induced by ischemia and diabetes were mainly NF-κB pathways-dependent. Overall, these findings indicated that inhibition of IL-17C might be a novel therapeutic strategy for DN and AKI.

Contributors

FW and JY designed and led the study; FW, JY, and FZ drafted the manuscript; JY, FW, FZ, GZ, SL, YK, LL, and YW performed the experiments; FW, JY, and NW performed data analysis; and XC provided reagents and technical support. All authors read and approved the final version of the manuscript. FW and JY have verified the underlying data. FZ and JY contributed equally.

Data sharing statement

No publicly created datasets or code are available in the current study. More anonymized data of the present study are available upon reasonable request to the corresponding authors.

Declaration of interests

The authors have declared that no conflict of interest exists.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2023.104607.

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