Increased interleukin-22 levels in lupus nephritis and its association with disease severity: a study in both patients and lupus-like mice model

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Abstract

Objective

Interleukin-22 (IL-22) has been considered as an inflammatory cytokine. In the present study, we investigated the potential role of IL-22 in lupus nephritis (LN).

Methods

We examined the IL-22 levels of serum and kidney tissue from LN patients and MRL/lpr mice. An intraperitoneal injection of saline, isotype control antibody (IgG), prednisone (3mg/kg/mouse), or anti-IL-22 mAb (5μg/kg/mouse or 25μg/kg/mouse) was administered twice a week from 6 to 18 weeks of age.

Results

IL-22 levels in both serum and kidney were significantly higher in LN patients as compared with those in healthy controls. The serum and renal levels of IL-22 in MRL/lpr mice were significantly increased over time. After MRL/lpr mice were treated with anti-IL-22 monoclonal antibody (mAb) for 12 weeks, significantly less urine protein and lower serum levels of creatinine and urea nitrogen were found. In addition, less renal injury score and few number of inflammatory cells per glomerulus were observed in MRL/lpr mice treated with anti-IL-22 mAb as compared with control groups.

Conclusions

Our results suggest that IL-22 as a pathogenic cytokine might be a potential target for treatment of lupus nephritis.

Key words

interleukin-22, systemic lupus erythematosus, lupus nephritis

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Received on April 6, 2018; accepted in revised form on June 29, 2018.

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Funding: this work was supported by grants from the National Natural Science Foundation of China (no. 81571578 to X. Yang), and Zhejiang Provincial Natural Science Foundation (no: Y15H100006 to X. Yang).
Competing interests: none declared.

Introduction

Lupus nephritis (LN) is seen in up to 60% of patients with systemic lupus erythematosus (SLE), and is associated with increased morbidity and mortality (1). Even with current immunosuppressive regimens, chronic renal insufficiency develops in about a quarter of patients. Although the hallmark in the pathogenesis for LN is production of autoantibody, complement activation, and immune-complex deposition with subsequent infiltration by inflammatory cells in kidney tissue (2-6), the exact mechanisms that lead to LN is still unclear. Thus, identification of crucial effectors which are correlated with disease severity of LN would be of great prognostic value, and be helpful for providing targets in treatment of LN.

Interleukin-22 (IL-22) is an IL-10 family cytokine member mainly produced by T helper (Th) cells (7). IL-22, which is preferentially released under Th-17-polarised conditions, signals through a class 2 receptor (IL-22R) composed of the subunits IL-22R1 and IL-10R2 (8, 9). The functional IL-22R1 is restricted to the nonhematopoietic cells of the skin, intestine, liver, lung and kidney (10). IL-22 is considered to have an exclusive function to translate antigen-specific immune responses only into tissue cells (11). Recently, numerous studies regarding the role of IL-22 in autoimmune diseases are emerging, and several reports suggest that IL-22 plays a critical role in the inflammation and proliferation cascade of various autoimmune diseases like SLE (11-14), rheumatoid arthritis (RA) (15-19), Sjögren's syndrome (SS) (20, 21) and psoriasis (7, 22).

Although evidence for the role of IL-22 is accumulating, this has not been put in the context of response to target IL-22 treatment in LN. Increased knowledge of IL-22 in LN may contribute to further understanding of the pathogenesis and lead to the development of new treatment strategies. Here we studied serum and kidney levels of IL-22 in LN patients and mouse models. We assessed the disease activity and histopathological findings in mouse models after treatment with anti-IL-22 monoclonal antibody.

Methods

Patients and samples

Thirty LN patients were recruited from April 2013 to December 2015 at the Department of Nephrology and Rheumatology of the Second Affiliated Hospital, Zhejiang University, Collage of Medicine. The study protocol was approved by the Ethics Review Committee of the Hospital (no. 2013-102) and was conducted in accordance with the principle set forth under the 1989 Declaration of Helsinki. All the subjects were given informed consents for the collection of peripheral blood and renal tissue. All patients fulfilled the American College of Rheumatology (ACR) diagnostic criteria of SLE (23) and was defined by renal biopsy. Disease activity was assessed by an independent physician using the SLE Disease Activity Index (SLEDAI) (24). Renal activity of SLE was assessed by renal SLEDAI score, which consisted of proteinuria, urinary casts, haematuria and pyuria of the original SLEDAI score. Fifteen healthy volunteers who had no any autoimmune disease and active infection served as controls. Ten normal renal tissues from para-carcinoma tissues as healthy controls (HCs) were confirmed by light microscope and immunofluorescence examination. Serum obtained from all subjects by centrifugation of peripheral blood samples were stored at -80°C until detection of cytokines. Renal biopsy was handled under ultrasound localisation. Renal tissue was extracted for histopathological assessment, RNA isolation and storage buffers for immunohistochemical analysis.

Animal experiments

All animal experiments were performed with approval of the Animal Ethics Committee of Zhejiang University, which complies with the Guide for the Care and Use of Laboratory Animals, 8th edition published by the US National Institutes of Health (NIH Publication, 2011). Fas-deficient MRL/lpr female mice at 5-6 weeks old were used as the model of lupus. They were obtained from Shanghai Slac Laboratory Animal CO. LTD (Shanghai, China), and housed in a specific pathogen free condition. To examine the pathogenesis of LN, MRL/lpr mice were randomly divided

into 3 groups (n=8 per group). Blood, urine and renal tissue were collected when MRL/lpr mice were 6, 12 and 18 weeks, respectively. In the experiment of treatment, MRL/lpr mice were randomly assigned to control or treatment groups (n=12 per group). Anti-IL-22 mAb (clone Y51A) was a kind gift from Dr Zheng SG (USC, Los Angeles, CA) (23). An intraperitoneal injection (i.P) of saline, isotype control antibody(IgG), prednisone (3mg/kg/mouse), or anti-IL-22 mAb (5μg/kg/mouse or 25μg/kg/mouse) was administered twice a week, starting at age of 6-weeks old. Blood and urine were collected once every 2 weeks. The mice were sacrificed at 18 weeks. Kidneys were harvested into appropriate storage buffers before further analysis.

Enzyme-linked immunosorbent assay (ELISA)

The amounts of IL-22 and IL-17 in human serum and IL-22 in mouse serum were detected with ELISA Kits (eBioscience) according to the manufacturer's instructions.

Assessment of proteinuria and renal function

Blood urea nitrogen (BUN) was determined by the Quantichrom DIUR 500 kit (BioAssay Systems, Hayward, CA). Serum and urinary creatinine were measured by the QuantiChrom Creatinine Assay Kit (BioAssay Systems). The ratio of albumin to creatinine in urine was measured using an ELISA kit (Exocell).

Renal histopathology

The kidney biopsy specimens were stained with hematoxylin and eosin (H& E), or periodic acid-Schiff (PAS). Renal histological morphology was observed by light microscopic examination. The kidney biopsy specimens were evaluated according to the International Society of Nephrology pathologic classification of LN (25). The pathological indices of activity of each biopsy specimen were determined by standard methods (26). The activity index (AI) is the sum of manual scores (0-3 each) of the following six parameters: endocapillary proliferation, leukocyte infiltration, hyaline deposits/wire loop, interstitial inflammation, necrosis and cellular crescents. The total score of AI is 0–24. The number of cells per glomerulus were counted in the PAS stained slides, using Image-Pro Plus 6.0.

Immunohistochemical analyses

Kidney tissues obtained from patients and MRL/lpr mice were fixed with 4% paraformaldehyde solution overnight at 4°C, dehydrated with a graded series of alcohol, washed, embedded in paraffin, and cut into 5μm thick sections. The sections were depleted of endogenous peroxidase activity by adding methanolic H_2O_2 for 10 minutes and blocked with normal serum for 30 minutes. After overnight incubation at 4°C with polyclonal antibodies against IL-22 and IL-17 (Abcam), the samples were incubated for 30 minutes with the secondary antibody, biotinylated anti-rabbit IgG, and incubated with streptavidin–peroxidase complex (Vector) for 1h, followed by incubation with 3,3'-diaminobenzidine (Dako) for 5 minutes. The sections were counterstained with haematoxylin. The samples were photographed using an Olympus photomicroscope.

Quantitative real-time PCR

Total RNAs were purified from kidney specimen of patients and mice and whole 293 cells using TRIzol reagent under manufacturer's protocol (Invitrogen) at RNase-free environment. RNA was used in a 20 μl reverse transcription system with First-Strand cDNA Synthesis Kit (GE Healthcare, Piscataway, NJ, USA). Quantitative RT-PCR was performed with the ABI Fast Dx Real-Time PCR instrument (Applied Biosystems, Thermo Fisher Scientific, MA, USA). IL-22 (human) primers: forward: 5´ CCAGGCTCAGCAACAGGCTAA 3´, reverse: 5´ TTTCAGCTTTGCTCTGGTCAAATG 3´; IL-22 (mouse) primers: forward: 5´ CAACTTCCAGCAGCCATACA 3´, reverse: 5´ GTTGAGCACCTGCTTCATCA3´; IL-17 primers: forward: 5´ CCTCAAGTTCCACTT 3´, reverse CACCAGCATCTTCTCCAC. The relative gene quantification was done using the 2^{-DDCt} method following normalisation to internal control glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Statistical analysis

Data was analysed using SPSS 22.0 and Graph Pad Prism 6, and presented as mean ± standard deviation. For experiments with only two groups, significance was determined by a Student's *t*-test or the Mann-Whitney U-test according to whether the distribution is normal or not. Significance for ≥2 groups was determined by one-way analysis of variance (ANOVA). *Post hoc* least significant difference (LSD) tests, in which all groups were tested against a control group as a reference, were performed if the results of the initial analysis of variance were significant. *p*-values <0.05 were considered significant and marked with one asterisk, while the *p*-values of <0.01 or <0.001 were marked with two or three asterisks.

Results

Clinical and demographic characteristics of subjects

The kidney biopsy specimens were evaluated according to the International Society of Nephrology pathologic classification of LN (25). Thirty patients consisted of 17 class IV LN, 6 class V LN, and 7 class II LN. The clinical and demographic features of LN patients and HCs are summarised in Table I. Age and gender ratio were comparable between LN patients and HCs (33.0±10.5 *vs*.31.4±11.7; 25/5 *vs*. 10/5; *p*>0.05)

IL-22 levels in serum and kidney tissue of LN patients

Serum levels of IL-22 in LN patients were significantly increased compared to those in HCs (234±120 pg/ml *vs*. 155 ± 118 pg/ml, *p*=0.046) (Fig.1a). Serum levels of IL-22 in class IV LN patients (292±108 pg/ml, n=17) were significantly increased compared to HCs (155±118 pg/ml, n=15, *p*=0.001), class II LN patients (153±80 pg/ml, n=7, *p*=0.008) and class V LN patients (162±85 pg/ml, n=6, *p*=0.018). There was no significant difference in serum levels of IL-22 among class V LN patients, II LN patients and HCs (Fig.1b). To examine the expression of IL-22 in kidney tissue of LN patients, the mRNAs coding for IL-22 were quantified by RT-PCR. The relative amount of IL-22 mRNAs in kidney tissue of LN patients was about

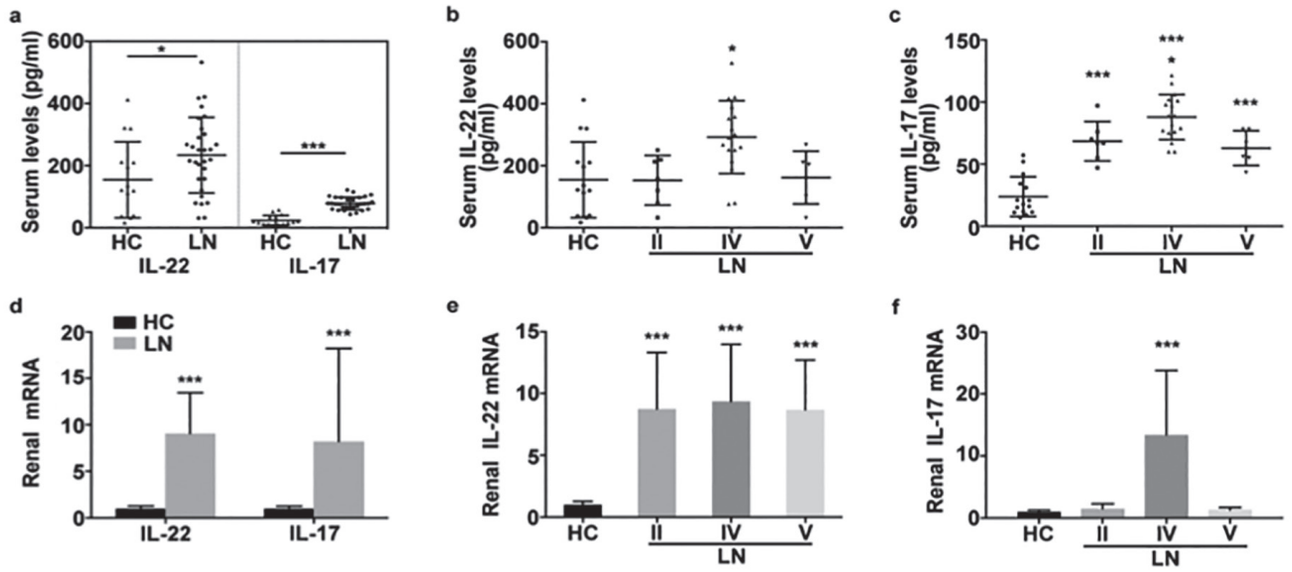


Fig. 1. Serum and renal levels of IL-22 in LN patients.
Serum levels of serum IL-22 and IL-17 of LN patients (n=30) and healthy controls (HCs, n=15) were measured by ELISA. (**a**) Serum levels of IL-22 and IL-17 in LN patients were significantly higher than those in HCs (Mann-Whitney U-test, *p<0.05, ***p<0.001). (**b**) Serum levels of IL-22 in class IV LN patients were significantly higher than those in patients with class V LN, class II LN and HCs (*p<0.05). (**c**) Serum levels of IL-17 in all 3 class LN patients was significantly higher than those in HCs (***p<0.001), and patients with class IV LN had significantly higher serum levels of IL-17 than patients with class V LN and class II LN (*p<0.05). Relative mRNA expression levels of IL-22 and IL-17 in kidney tissue were quantified by RT-PCR. (**d**) Renal IL-22 and IL-17 mRNAs in LN patients were significantly higher than those from HCs (student's t-test, ***p<0.001). (**e**) Renal IL-22 mRNAs in all 3 class LN patients were higher than those in HCs (***p<0.001). (**f**) Renal IL-17 mRNAs in class IV LN patients were higher than those in class II, V LN patients and HCs (***p<0.001). Statistical analysis of **b**, **c**, **e** and **f** was performed by one-way ANOVA and LSD tests.

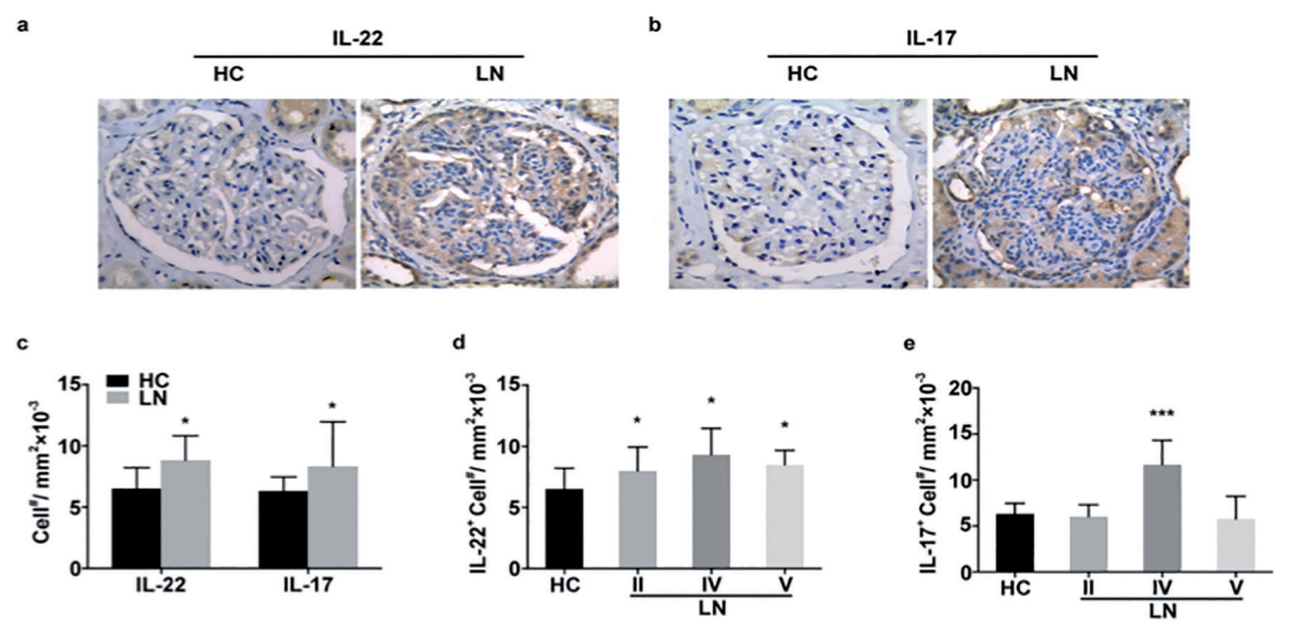


Fig. 2. Expression of IL-22 and IL-17 in renal tissue of LN patients.
(**a, b**) Expression of IL-22 and IL-17 in kidney tissue of healthy controls (HCs) and LN patients were assessed by immunohistochemistry. Quantification of IL-22$^+$ and IL-17$^+$ cells in Fig. a, b. (**c**) Expression of both IL-22 and IL-17 on renal biopsies from LN patients were significantly higher than those from HCs (Student's t-test, *p<0.05). (**d**) All classes of LN patients had significantly more IL-22$^+$ cells on renal biopsies than HCs (*p<0.05). (**e**) class IV LN patients had significantly more IL-17$^+$ cells on renal biopsies than class II, V LN patients and HCs (***p<0.001). Statistical analysis of **d** and **e** was performed by one-way ANOVA and LSD tests.

9-fold higher than that in HCs (p<0.001, Fig. 1d). Renal IL-22 mRNAs were not significantly different among 3 classes of LN patients (p>0.05) and they were higher than those of HCs (p<0.001) (Fig. 1e). Meanwhile, the expression levels of IL-22 and IL-17 in kidneys of LN patients were assessed by immunohistochemistry (Fig. 2a, b). IL-22$^+$ and IL-17$^+$ cells were quantified. Quantification of IL-22$^+$ cells on renal biopsies from LN patients was significantly increased compared with that from HCs (p<0.05) (Fig. 2c). There was no significant difference in quantification of IL-22$^+$ cells

Table I. Clinical and demographic features of patients with lupus nephritis (LN).

Characteristics	LN (n=30)	HC (n=15)
Age, years	33.0 ± 10.5	31.4 ± 11.7
Female, n (%)	25 (83.3%)	10 (66.7%)
Male, n (%)	5 (16.7%)	5 (33.3%)
Diseases duration (years)	1.3 ± 1.1	NA
Malar rash, n (%)	22 (73.3%)	NA
Arthritis, n (%)	26 (86.7%)	NA
Serositis, n (%)	16 (53.3%)	NA
CNS involvement, n (%)	5 (16.7%)	NA
Haematological involvement, n (%)	23 (76.7%)	NA
Serum C3 levels (mg/dl)	53.1 ± 32.4	NA
Serum C4 levels (mg/dl)	11.6 ± 10.9	NA
Anti-dsDNA levels (U/ml)	227.4 ± 31.5	NA
Urinary protein (gm/24 hr)	3.22 ± 1.9	NA
Ccr (ml/24 hr)	63.5 ± 33.1	NA
SLEDAI score	15.8 ± 3.5	NA
Renal SLEDAI score	9.8 ± 3.0	NA
Histological activity index(AI)	6.7 ± 3.1	NA
Histological chronicity index(CI)	2.8 ± 1.5	NA
Dose of oral corticosteroids (mg/day)	30.6 ± 7.9	NA
Hydroxychloroquine, n (%)	27 (90.0%)	NA
Mycophenolate mofetil, n (%)	13 (43.3%)	NA
Cyclophosphamide, n (%)	7 (23.3%)	NA

HC: healthy control; CNS: central nervous system; C3: complement 3; C4: complement 4; Anti-dsDNA: anti-double stranded DNA antibody; Ccr: creatinine clearance rate; SLEDAI: systemic lupus erythematosus (SLE) disease activity index; NA: not applicable.

among 3 classes of LN patients ($p>0.05$) and they were all more than those of HCs ($p<0.05$, Fig. 2d).

Significantly increased levels of IL-17 were seen in LN patients (78±20 pg/ml, n=30) compared to HCs (24 ± 16 pg/ml, $p=0.000$) (Fig.1a). Serum levels of IL-17 in class IV (88±18 pg/ml), class V (62.8±14 pg/ml) and class II (68±16 pg/ml) were significantly higher than HCs (respectively, $p=0.000$) (Fig.1c). Serum levels of IL-17 in class IV LN patients were significantly increased compared to those in class II LN patients ($p=0.013$) and class V LN patients ($p=0.003$), while there was no significant difference in serum levels of IL-17 between LN patients of class V and class II ($p=0.551$) (Fig.1c). The relative amount of IL-17 mRNAs in kidney tissue of LN patients was about 8-fold higher, than that in HCs ($p<0.001$, Fig. 1d). Only class IV LN patients had significantly higher renal IL-17 mRNA than HCs ($p<0.001$, Fig. 1f), while renal IL-17 mRNA levels in class II and V LN patients were similar to those in HCs ($p>0.05$, Fig. 1f). Consistently, only class IV LN patients had more IL-17$^+$ cells on renal biopsies than HCs ($p<0.001$, Fig. 2e), while Class II and V LN patients were not significantly different from HCs in the quantification of IL-17 ($p>0.05$, Fig. 2e).

IL-22 levels in serum and kidney tissue of MRL/lpr mice

To further confirm the expression of IL-22 in LN, animal experiment of MRL/lpr mice that spontaneously develop severe form of lupus -like disease with growth of age (27) were examined. The results showed that serum levels of IL-22 in MRL/lpr mice were gradually increased from 6 to 18 weeks of age. Serum levels of IL-22 in MRL/lpr mice at 12 weeks significantly increased compared to those at 6 weeks ($p<0.01$, Fig.3a), and serum levels of IL-22 in MRL/lpr mice at 18 weeks were significantly higher than those at 6 and 12 weeks ($p<0.001$, Fig.3a). Renal levels of IL-22 mRNA in MRL/lpr mice were dramatically increased with growth of age. Renal levels of IL-22 mRNA in MRL/lpr mice at 12 and 18 weeks significantly increased compared to those at 6 weeks ($p<0.001$, Fig.3b), and renal levels of IL-22 mRNA in MRL/lpr mice at 18 weeks were significantly higher than those at 12 weeks ($p<0.001$, Fig. 3b). Immunohistochemistry staining of kidney sections also showed the significant increase of IL-22 expression in

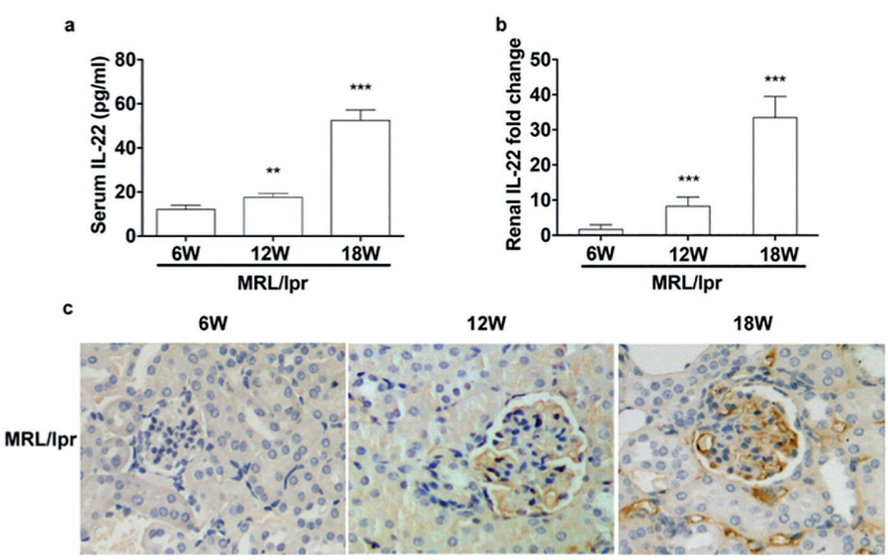


Fig. 3. IL-22 levels in serum and kidney tissue of MRL/lpr mice.
Serum levels of IL-22 in MRL/lpr were examined by ELISA. (**a**) Serum levels of IL-22 in MRL/lpr mice at 12 weeks significantly increased compared to those at 6 weeks (n=8, $^{**}p<0.01$), and serum levels of IL-22 in MRL/lpr mice at 18 weeks were significantly higher than those at 6 and 12weeks (n=8, $^{***}p<0.001$). Renal levels of IL-22 mRNA were determined by RT-PCR. (**b**) Renal levels of IL-22 mRNA in MRL/lpr mice at 12 and 18 weeks significantly increased compared to those at 6 weeks (n=8, $^{***}p<0.001$), and renal levels of IL-22 mRNA in MRL/lpr mice at 18 weeks were significantly higher than those at 12 weeks ($^{***}p<0.001$). Statistical analysis of a, b was performed by one-way ANOVA and LSD tests. (c) Immunohistochemistry study showed the presence of IL-22 (brown colour) in kidney tissue of MRL/lpr mice was significantly increased with the development of nephritis.

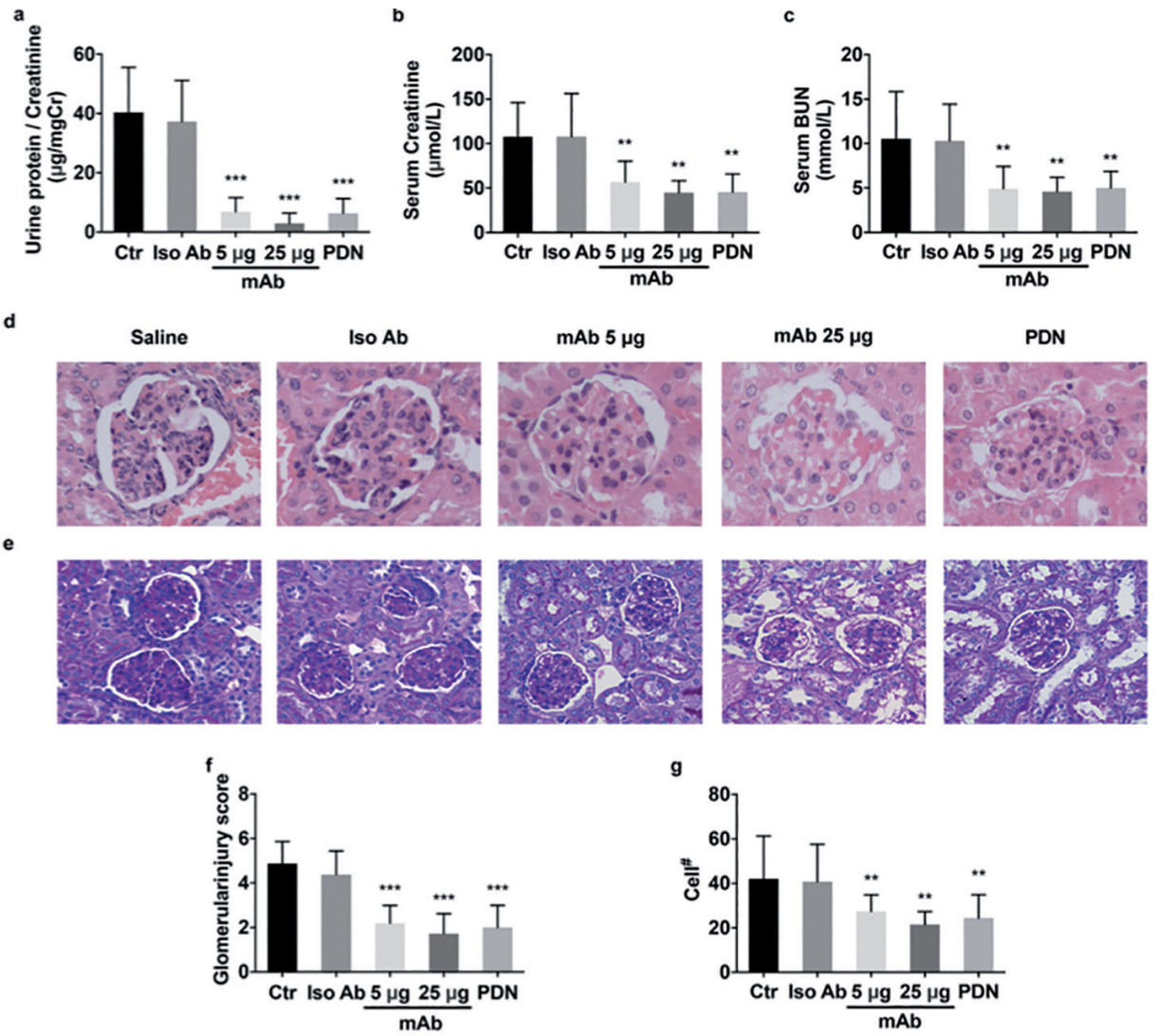


Fig. 4. Effects of anti-IL-22 mAb treatment on kidney disease of MRL/lpr mice.
Renal function in MRL/lpr after treatment of anti-IL-22 mAb. (**a**) Proteinuria levels were analysed by ELISA, normalised to urine creatinine to adjust for variations in urine output, at 18weeks. Results showed that MRL/lpr treated with anti-IL-22 mAb (5 or 25μg/kg BW) or prednisone (PDN) had significantly less quantity of proteinuria compared with those treated with saline (Ctr) or isotype IgG (IsoAb) (***p<0.001). (**b**, **c**) MRL/lpr mice treated with anti-IL-22mAb or prednisone had significantly lower levels of serum creatinine and blood urea nitrogen (BUN) in blood as compared with those treated with saline or isotype IgG (**p<0.01). Renal histopathology in MRL/lpr after treatment of anti-IL-22 mAb. Kidneys were harvested and the sections were stained for H & E (**d**), and PAS-staining (**e**). Quantification of renal injury scores and cell numbers per glomerulus in Fig. **e**. MRL/lpr mice treated with anti-IL-22 mAb or prednisone had significantly less renal injury score (***p<0.001) (**f**), and fewer cell numbers (**p<0.01) (**g**) compared to those treated with saline or isotype IgG. Statistical analysis of **a**, **b**, **c f** and **g** was performed by one-way ANOVA and LSD tests.

MRL/lpr mice with the development of nephritis (Fig. 3c).

Urine protein, serum creatinine and blood urea nitrogen levels in MRL/lpr mice after treatment of anti-IL-22 mAb

To define the role of IL-22 in the pathogenesis of LN, anti-IL-22mAb was injected i.P. twice a week into MRL/lpr mice at 5-6 weeks of age, *i.e*. 1-2 weeks prior to nephritis onset, for 12 weeks. Urine protein (p<0.001, Fig. 4a), serum creatinine (p<0.01, Fig. 4b) and blood urea nitrogen (BUN) (p<0.01, Fig. 4c) were all significantly reduced in MRL/lpr mice at 18 weeks after treated with anti-IL-22 mAb or with prednisone as compared with saline- or isotype IgG-treated group. Treating mice with different dosages of anti-IL-22 mAb (5 or 25μg/kg BW) resulted in similar effect on reduction of proteinuria, creatinine and blood urea nitrogen (p>0.05).

Renal activity index in MRL/lpr mice after treatment of anti-IL-22 mAb

The pathological changes in kidneys of MRL/lpr mice were analysed after anti-IL-22 mAb treatment. Morphologically as shown by H&E staining, there was significantly less renal injury in MRL/lpr mice treated with anti-IL-22 mAb or prednisone than those treated with saline or isotype IgG (Fig. 4d). At 18 weeks of age, more glomerular cellularity, collapse of capillary lumina, and thicker basement membranes were observed in the control MRL/lpr mice as shown by PAS staining (Fig. 4e). In contrast, the renal AI score (p<0.001, Fig. 4f) and number of cells per glomerulus (p<0.01, Fig.4g) were significantly lower in

MRL/lpr mice treated with anti-IL-22 mAb (5 or 25μg/kg BW) or with prednisone than those treated with saline or isotype IgG.

Discussion

LN is seen in a significant proportion of SLE patients and associated with a poor prognosis. However, the pathogenesis of LN is unclear. Our present studies demonstrated increased IL-22 levels both in blood and kidney tissue of LN patients and MRL/lpr mice. Blocking IL-22 with anti-IL-22 mAb inhibited the progression of LN in mouse model. These data revealed that IL-22 plays an important role in the pathogenesis of LN.

There are several studies about the expression of IL-22 in periphery blood of SLE patients, however their results were inconsistent (12-15). Our previous study showed the difference in serum IL-22 levels between SLE patients with LN and skin disease (15), suggesting the expression of IL-22 in blood may be variant depending on organs involvemened of SLE patients. In the present study, we focused on the expression of IL-22 in LN patients. Our results demonstrated increased IL-22 levels in blood and kidneys of LN patients. According to the pathogenic forms of LN, we found that class IV LN patients had significantly higher expression of IL-22 both in serum and kidney tissue than normal controls, however, Class II and V LN patients had higher expression of IL-22 only in kidney tissue. These results indicated serum expression of IL-22 may vary with different pathogenical forms of LN, suggesting serum IL-22 levels cannot be representative of the IL-22 status in LN patients. This phenomenon may be mainly associated with the production of IL-22 and the pathological forms of LN. IL-22 is mainly produced by T helper cells (8,9). Th cells, including Th17 and Th22 cells, were found to have the capacity to express IL-22 in SLE patients (14,15). IL-22 can be produced by both systemic and local IL-22-producing Th cells. Th cells are important mediators in both mouse models and human patients (3), and kidney-infiltrating Th cells are activated and express a wide array of proinflammatory cytokines in the pathogensis of LN (28,29). In our study, only increased renal IL-22 in class II and V LN patients indicated that renal IL-22 would be mainly produced by local kidney-infiltrating Th cells. So, serum IL-22 levels were not increased in class II and V LN patients. According to the pathologic classification, which identifies mesangial involvement as class II, proliferative disease as class IV and membranous disease as class V LN (25), kidney-infiltrating Th cells are predominantly present in kidneys of class IV LN and maintain high production of cytokines (3). Then, abundant kidney-infiltrating Th cells may result in more quantity of IL22 in kidneys of class IV LN, then overexpressioned IL-22 may leakage into the vascular compartment. In addition, there may be increased IL-22-producing Th cells in the peripheral blood of class IV LN patients. So, class IV LN patients had higher serum levels of IL-22 than class II and V LN patients and healthy controls.

IL-17 is the main cytokine from Th17 cells and plays a central role in the pathogenesis of LN (30). We also found increased expression of IL-17 in serum and kidneys of LN patients. Our results were consistent with previous studies (28, 31). In addition, we found that the expresson of IL-17, such as IL-22, in both serum and kidneys of class IV LN patients was increased, and in contrast to IL-22, the expresson of IL-17 was increased only in serum but not in kidneys of class II and V LN patients. Therefore, the expression of IL-17 and IL-22 may depend on pathological pattern in LN.

In the mouse model of lupus-prone MRL/lpr, we found the levels of IL-22 expression in serum and kidney tissue were increased at the occur of LN when MRL/lpr mice was at 12 weeks. Up to 18 weeks of MRL/lpr mice, renal pathology showed severe kidney disease, and the higher levels of IL-22 in serum and kidney tissue were found, suggesting IL-22 is associated with the disease severity of LN. This study of mouse models futher confirmed that IL-22 may be involved in the pathogenesis of LN.

IL-22 is believed to be a critical modulator in various inflammatory diseases with a broad range of bioactivities on diverse target cells (32). IL-22 has dual pro-inflammatory and anti-inflammatory nature in chronic inflammatory diseases, depending on the nature of the affected tissue and the local cytokine milieu (33). Psoriatic patients showed highly elevated IL-22 plasma levels, which correlated with the disease severity (34). IL-22 synergised with other cytokines, such as TNF-α, IL-17 and IL-20, to form a cytokine network that orchestrates the progression of many different pathogenic features of psoriasis (35). Blocking IL-22 can both affect keratinocyte dysregulation and neutrophil infiltration in a mouse model with psoriasis skin inflammation (36). So, IL-22 plays a pathogenic role in psoriasis. In RA, IL-22 promoted osteoclastogenesis by induction of receptor activator of nuclear factor kappa-B ligand (RANKL) in human synovial fibroblasts (19). However, another study showed that IL-22 reduces the severity of collagen-induced arthritis, when administered prior to the onset of the disease, the mechanism of which is associated with increased levels of IL-10 (37). These findings suggest that IL-22 has dual functions, *i.e.* protective or pathogenic, in inflammatory arthritis, depending on the different phases of the disease development. IL-22 was able to induce mRNA expression of acute phase protein such as serum amyloid A (SAA),α1-antichymotrypsin, and haptoglobin in the HepG2 human hepatoma cell line and concordantly, an increase of SAA mRNA expression in the liver of IL-22 treated mice (38). The administration of anti-IL-22 antibody resulted in incipient liver necrosis during *Salmonella enteritidis*- infected p35-deficient mice (39). IL-22 is generally considered to be protective in liver diseases.

To assess the role of IL-22 in LN, MRL/lpr mice was treated with anti-IL-22 mAb in the mouse model of lupus. Our studies showed that administration of anti-IL-22mAb for MRL/lpr mice, prior to the onset of LN, significantly reduced less proteinuria, better renal function, less cellular infiltration in the renal pathology. The efficacy of anti-IL-22mAb is equal to that of prednisone currently considered as an effective therapeutic for LN. These results suggest that IL-22 might be a pathogenic cytokine in LN. It was found that kidney cells are

important target cells of IL-22 (32). IL-22 binding to IL-22R complex leads to a cascade of downstream signalling pathways (28). Initial studies utilising a murine kidney cell line revealed that IL-22R ligation induced phosphorylation of STAT3, and, to a lesser extent, STAT5, while other studies observed phosphorylation of STAT1, STAT3, and STAT5 in a human kidney cell line (38). We assume that IL-22 plays a pathogenic role possibly through regulating STAT3 signalling and inducing the production of pathogenic cytokine

Conclusion

Our results suggest IL-22 was involved in pathogenesis of LN. Our study demonstrates the benefits of anti-IL-22 mAb treatment for murine LN with minimal risk of kidney disease. Further understanding the functional mechanism of IL-22 would certainly be beneficial in the future treatment of LN and other autoimmune diseases.

Acknowledgments

We thank Biqin Tan for performing the cell culture, Jian Ma for technical assistance and Prof. Hong Yu for revising the article.

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