IJKD KIDNEY DISEASES

TACI Ig Fusion Protein Inhibits TLR4/MyD88/NF-κB Pathway Alleviates Renal Injury in IgA Nephropathy Rats

Jianhua Sun,[1,#] Yue Cheng,[1,#] Wei Zhang,[2] Peng Chen,[2] Xinnan Chen,[3] Caili Wang,[3] Zengyan Li[3,*]

[1]Department of Nephrology, Baotou Medical College, Baotou, China.
[2]Department of Pathology, The First Affiliated Hospital of Baotou Medical College, Inner Mongolia University of Science and Technology, Baotou, China.
[3]Department of Nephrology, The First Affiliated Hospital of Baotou Medical College, Inner Mongolia University of Science and Technology, Baotou, China.
[#]Jianhua Sun and Yue Cheng contributed equally to this work

Keywords. TACI Fusion Protein; IgA Nephropathy; Rats; Inflammatory Damage

Original Paper

Introduction. To evaluate the impact of TACI fusion protein (TACI-Ig) on IgA nephropathy (IgAN) in rats, and to explore its mechanism and relationship with TLR4/MyD88/NF-κB pathway.
Method. Sprague Dawley (SD) rats were divided into six groups: control, model, TACI-Ig low dose (TACI-Ig-L), medium dose (TACI-Ig-M), high dose (TACI-Ig-H), and prednisone acetate (PAT) group. The control group and model group received physiological saline injections, while the TACI-Ig groups were administered doses of 7.18, 14.36, and 28.72 mg/kg of TACI-Ig, respectively. PAT group was pretreated with prednisone acetate. After 8 weeks, kidney weight/body weight ratios, 24-hour urine protein (24 h UP), serum creatinine (SCr), and blood urea nitrogen (BUN) levels were measured. Additionally, concentrations of B cell activating factor (BAFF), APRIL, and Gd-IgA1 were evaluated by using ELISA. Pathological changes in kidney tissues were scored, and TLR4, MyD88, NF-κB expression levels were detected through western blot (WB) and RT-qPCR.
Results. Renal function assessments showed that the IgAN model group exhibited increased in 24 h UP, SCr, BUN, and elevated serum levels of BAFF, APRIL, Gd-IgA1, alongside higher TLR4/MyD88/NF-κB protein expression. TACI-Ig treatment significantly reduced proteinuria, SCr, BUN, levels of BAFF, APRIL, and Gd-IgA1 in IgAN rats. Pathologically, TACI-Ig ameliorated glomerular mesangial deposition and fibrosis. It also inhibited TLR4/MyD88/NF-κB protein expression, demonstrating anti-inflammatory and immune regulatory effects.
Conclusions. TACI-Ig mitigates renal injury in IgAN rats by reducing inflammatory infiltration and IgA deposition and suppressing the pathway of TLR4/MyD88/NF-κB, offering data for developing effective treatments for IgAN.

IJKD 2024;18:326-36
www.ijkd.org
DOI: 10.52547/ijkd.8173

INTRODUCTION

Primary IgAN is the most common type of glomerulonephritis in Asian populations and is characterized by hematuria and mild proteinuria.[1] Clinical diagnosis of IgA nephropathy (IgAN) is usually confirmed by renal biopsy.[2] At present, although the pathogenesis of IgAN has been deeply explored in relevant studies, its regulatory mechanism and pathophysiology are still lacking a comprehensive understanding. The regulation of humoral immunity by B cells is inseparable from B lymphocyte stimulating factor (BlyS/BAFF)

and proliferation-inducing ligand (APRIL), which regulate the expression of survival promoting genes through these two mediators to maintain humoral immunity.[3] Studies have found that the occurrence of a variety of diseases, especially autoimmune diseases, can cause a substantial increase in serum BAFF level[4,5] In addition, overexpression of BAFF in transgenic mice has been found to significantly increase the immune complex of B lymphocytes in peripheral blood of mice.[6] BAFF binds to three specific receptors, including BAFF-R, TACI, and BCMA. TACI is a calcium regulator, which can strengthen the interaction between protein ligands. BCMA is a mature protein. These three receptors have been shown to play a decisive role in the survival of B cells.[7] The TLR4/MyD88/NF-κB signaling pathway is a classic inflammatory pathway, which plays an important role in the pathological process of various inflammatory diseases, and is also closely related to IgAN.[8] The passive activation of TLR4 causes dimerization of the TIR domain in TLR4 cells and recruitment of MyD88 and MAL (MyD88 adaptor like), resulting in activation of the NF-κB pathway. This pathway promotes the phosphorylation of irak and regulates the signal transduction process.[9-11] After binding to receptors, BlyS and APRIL can drive TLR and activate NF-κB, JNK, MAPK and other pathways to regulate cell proliferation, differentiation and maturation. The TACI receptor can bind to MyD88 to induce B cells to produce IgA.[9] Clinical Studies have shown that TLR stimulation in ddY mice prone to IgAN can increase serum levels of abnormal Gd IgA1, indicating its involvement in the pathogenesis of IgAN.[12] And studies have shown that TLR4 is elevated in IgAN patients and is associated with disease severity.[13]

TACI fusion protein (TACI-Ig) is a recombinant fusion protein that contains the Fc component of human IgG and the ligand binding domain of TACI receptors.[14] It is a BLyS/APRIL dual inhibitor, currently in Phase II clinical studies, and has been approved for the treatment of SLE patients (China) and successfully treated 1 case of membranous-type nephropathy.[16]

The TLR4/MyD88/NF- κ B pathway is at the heart of this study, and its regulatory role in the pathogenesis of IgAN allows for a clearer understanding of its influence on the progression of IgAN. By conducting an exhaustive analysis of these pivotal proteins, the study not only aims to elucidate the specific molecular mechanisms by which the TACI-Ig influences the disease process but also to explore its role in regulating immune response, reducing inflammation and restoring renal homeostasis as well as its indirect impacts on renal function and pathology.

The ultimate goal of this study is that by pioneering new therapeutic avenues and elucidating the molecular underpinnings of IgAN, this research could significantly advance our understanding of kidney disease, leading to enhanced patient care, improved treatment outcomes, and a better quality of life. In doing so, this study marks a critical advancement in the ongoing fight against renal diseases, setting a new benchmark for innovative research and therapeutic development in nephrology.

MATERIALS AND METHODS

Animals

Thirty-six Sprague Dawley (SD) rats, sterile grade, male, weighing 0.18-0.20Kg, purchased from the Department of Medicine (Experimental Animal Science) of Peking University, batch number: 11033222010068862. Animal license number: SCXK (Beijing) 2021-0013. The experimental plan was passed by Medical Ethics Committee of the First Affiliated Hospital of Baotou Medical College, Inner Mongolia University of Science and Technology (2023 Ethics Review (clin) No. (014)). The animals were kept in the Experimental Animal Center of Baotou Medical College, in a specially ventilated cage, in a room with constant temperature, humidity, and no pathogens. They circulated every 12 hours/dark and no restrictions on food or water. All animals are handled in strict accordance with the guidelines for the Care and use of Experimental Animals issued by the National Institutes of Health.

Reagents

BSA and LPS: Beijing Solebao Company, Beijing, China; batch number A8020-100g, L8880-10mg); Carbon tetrachloride (CCl4) and Castor oil purchased: Shanghai McLean Biochemical Technology Co., Ltd. (Shanghai, China), batch number: C805332, C805202; The BAFF, APRIL, Gd IgA1 enzyme-linked immunosorbent assay (ELISA) kits: Jiangsu Enzyme Immunoassay Industrial Co.,

Ltd. (Yancheng, China); The RNA extraction kit: Tiangen Biochemical Technology (Beijing, China) Co., Ltd., batch number: DP424; The reverse transcription kit: Baori Medical Biotechnology (Beijing, China) Co., Ltd., batch number: TAKARA-RR047A; The universal fluorescence quantitative PCR kit: Biosharp (Hefei, China), batch number: BL697A.

EXPERIMENTAL METHODS

Establishment of rat IgAN model

Male SD rats all weigh around 200 g. BSA was prepared into a solution with a concentration of 150 g/L, with a dose of 600 mg/Kg. It was orally administered the next day for 12 weeks; 0.3 ml of Castor oil + 0.1 ml of CCl4, subcutaneously injected once a week for 12 weeks; 0.05 mg of LPS was injected into the tail vein at weeks 6, 8, 10, and 12 until the end of the experiment. Immunofluorescence staining showed the deposition of IgA immune complexes in mesangial area, indicating successful modeling. The rats in each group were killed by intraperitoneal injection of 150 mg/kg of pentobarbital sodium.

Grouping of rats and drug intervention

All rats were randomly divided into six groups: control, model, TACI-Ig low dose (TACI-Ig-L), medium dose (TACI-Ig-M), high dose (TACI-Ig-H), and drug control group (prednisone acetate) 5 mg/kg. After the successful establishment of the IgAN model, the TACI fusion protein, produced by Rongchang Biopharmaceutical Co., Ltd. in Yantai, China, was administered at a dosage of 7.18 mg/kg in the low-dose group, 14.36 mg/kg in the medium dose group, and 28.72 mg/kg in the high-dose group. The dosage of rats was calculated according to the body surface area method in the drug instructions and pharmacology experimental methodology, and the proportion of TACI-Ig intervention dose was set. TACI-Ig was injected once every other day for a total of 8 weeks. The control group and model group were injected with the same dose of physiological saline until the end of the 20th week.

General situation and renal index of IgAN rats

Observed and recorded abnormal changes in physical signs such as weight, behavioral activity, and hair status of rats before and after administration. Both kidneys were accurately weighed by a precision balance, and the kidney index was calculated by the ratio of kidney mass to body weight.

Automatic biochemical analyzer for detecting renal function in rats

All rats were restricted from eating but allowed to drink water freely, and 24-hour urine was collected. Urine samples were centrifuged at 3000 r/min^{-1} to remove sediment, and the supernatant was collected, and the 24 h UP was measured by using the biuret method. Tail venous blood was taken from rats and centrifuged (4°C, 3000 r/min-1), and the supernatant was collected. Then, serum creatinine (SCr) and blood urea nitrogen (BUN) were measured by using a fully automated biochemical analyzer.

Sampling of rat kidney tissue

After eight weeks of treatment, according to the above-mentioned plan, serum was collected and placed at low temperature (-80°C) immediately for biochemical testing. The kidney tissue of appropriate size was cut and treated with 10% neutral formalin for subsequent histopathological examination. The remaining kidney tissue was stored at -80°C for subsequent experiments.

ELISA protein detection method

An ELISA kit was used to detect protein expression. Firstly, it was diluted to standard according to operation manual and added to the sample. Then incubated for 30 min (37°C), and diluted the detergent. The liquid was discarded from ELISA board. Each hole was then filled with detergent and discarded after 30 seconds. This process was repeated five times until the enzyme-linked immunosorbent assay (ELISA) plate was dried.

Fifty μL ELISA reagent were added to each hole and incubated at 37°C for 30 minutes, the ELISA plate was cleaned. After incubating in dark at the condition of 37°C for 15 minutes, the reaction was terminated. Next, the absorbance values of each well at a wavelength of 450 nm were detected.

Hematoxylin and eosin (H&E) staining of renal tissue

The kidney tissue samples were embedded

in paraffin and cut into sections with four μm. Afterwards, the stained sections were examined histopathologically by using light microscopy to observe the mesangial and matrix proliferation in the glomeruli.

High iodate Schiff staining of renal tissue (Periodic Acid Schiff, PAS)

The first two steps of PAS are similar to HE staining.The tissues were soaked with 1% periodate brine to achieve staining, washed with distilled water after 20 minutes, and reacted with Gachiff's reagent for 2 hours (37°C, away from light). We placed it in a 0.5% sodium bisulfite solution for 10 minutes, and then rinse three times before re-staining with hematoxylin. Finally, the renal tissue slices were observed under a light microscope

Immunofluorescence staining

The renal tissue was embedded and frozen sections were prepared, with slices cut to a thickness of 6μm. The slices were fixed with acetone at 4°C for 15 minutes and permeated with 0.5% citrate buffer. Subsequently, 5% goat serum was sealed at room temperature for 30 minutes and incubated overnight with primary antibody IgA (1:100) at 4°C. Then incubated with the anti-mouse secondary antibody at 37°C for 1 hour, and stained for 5 minutes. Finally, the immunofluorescence images were observed under a laser confocal microscopy.

Western blot protein detection

Frozen kidney tissue was treated with an efficient tissue lysis buffer containing PMSF and phosphatase inhibitors to obtain renal tissue homogenate. The nuclear protein extraction kit (Shanghai Beibo Biotechnology Co., Ltd., Shanghai, China) was used to extract nuclear and cytoplasmic proteins. The protein was separated from the sample using SDS-PAGE and transferred onto a polyvinylidene fluoride membrane. Then, the PVDF membrane was sealed with 5% skimmed milk for 1.5 hours, and the primary antibody was added for incubation (4°C), TLR4 (66350, 1:1000), MyD88 (67969, 1:2000), NF-κB p65 (AF5006, 1:1000), β-actin (21338, 1:5000). Then, goat anti mouse antibodies (SA00001-1, 1:5000) and goat anti rabbit antibodies (L3012, 1:5000) were used for incubation at room temperature. The chemiluminescence kit was used to visualize immunoreactive protein bands and then quantified by using Image J6.0 software. β-Actin serves as a load control for total protein.

RT qPCR detection

TRNzol Universal Total RNA Extraction Reagent (TIANGEN, DP424, Shanghai, China) was used to extract RNA from renal tissue, as well as being used as a template to generate cDNA through reverse transcription. The primers were sourced from Shanghai Shenggong (Shanghai, China). Real time fluorescence quantitative PCR strictly followed the instructions of the reagent kit, CT values from each group were collected, and real-time PCR analysis was performed.

Statistical analysis

SPSS 20.0 software (IBM, Armonk, NY, USA) was used for statistical analysis, $\bar{x} \pm s$ was used to describe econometric data and inter group comparisons were performed by using one-way ANOVA. The comparison of two means was performed by using t-test. $P < .05$ indicated statistically significant differences, while $P < .01$ indicates that the difference is statistically significant.

RESULTS

Effects of TACI fusion protein on general signs and renal index in IgAN rats

During the experimental modeling procedure, the rats in the model group, subjected to subcutaneous injection of CCl4, exhibited different degrees of extreme struggle and hair explosion, in contrast to the normal control group rats. After intravenous injection of lipopolysaccharide into the tail vein, symptoms of lethargy and urinary incontinence appeared, which relieved on the next day. None of these conditions were found in the other groups.

On the other side, one of the indicators of renal injury, Figure 1, the renal index of rats in model group increased compared to the normal control group. In the TACI-Ig drug intervention group, the renal index of rats in high-dose group was lower than the model group.

TACI fusion protein improves renal function and reduces renal pathological damage in IgAN rats by reducing urinary protein levels

The renal protective effect of TACI-Ig on a rat IgAN model was evaluated by detecting changes in 24 h

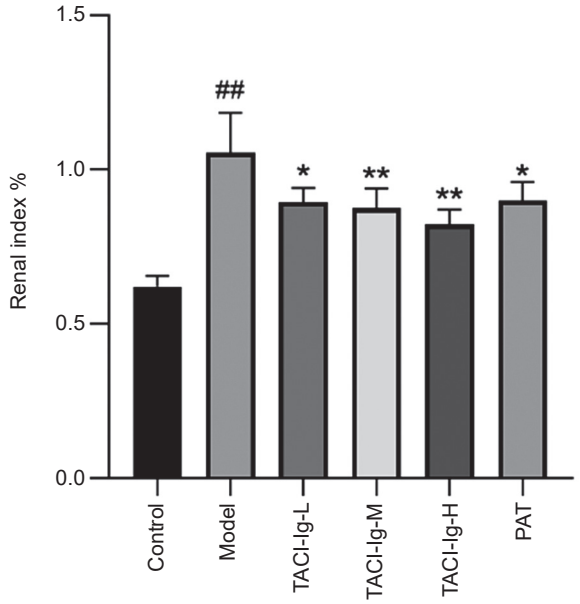

Figure 1. Effect of TACI-Ig on renal index in IgAN rats. Compared with the normal group, #*P* < .05, # #*P* < .01; Compared with the model group, **P* < .05, * * *P* < .01. Each group of samples has *n* = 5.

UP, serum creatinine, and blood urea nitrogen. The 24 h UP of the model group [(71.69 ± 13.39)] μg] was higher than normal control group [(15.88 ± 5.806)] μg], which with significantly increased (*P* < .01, Figure 2A). Compared to the IgAN model group, the 24 h UP in the TACI-Ig medium dose group [(43.87 ± 10.43) μg], TACI-Ig high dose group [(34.60 ± 6.855)] μg] and the drug control group [(46.65 ± 13.21)] μg] were all significantly decreased (*P* < .01). Although the 24 h UP [(52.18 ± 7.176)] μg] in the TACI-Ig low dose group decreased (*P* < .05), as compared with the IgAN model group; however, there was no statistically significant difference, in 24 h UP, between the drug control group and all TACI-Ig treatment groups.

As shown in Figure 2B-C, the serum creatinine and BUN in IgAN model group were higher than normal control group (*P* < .01). After treatment with TACI-Ig and prednisone acetate, the elevation of the above indicators reduced to varying degrees, with the decrease in TACI-Ig high dose group being the most significant (*P* < .01).

Figure 3 shows glomerular H&E and PAS staining. High dose of TACI-Ig could significantly reduce the glomerular mesangial proliferation than IgAN model group. Under electron microscope, high density electron deposition in the mesangial region of the glomerulus was observed and protruded into the renal capsule cavity. After TACI-Ig intervention, the mesangial high density electron density decreased in different degree. Compared with the TACI-Ig treatment group, the electron dense deposition in the drug control group was higher.

The above results indicate that TACI-Ig can effectively improve renal function and alleviate pathological damage of renal tissue.

The effect of TACI fusion protein on the production of BLyS factor, proliferation inducing ligand (APRIL), and galactose deficiency antibody IgA1 (Gd IgA1)

To further investigate the renal protective effect of TACI-Ig on IgAN rats, the expression of BLyS and APRIL factors in serum of IgAN rats were measured. The serum levels of BLyS and APRIL in

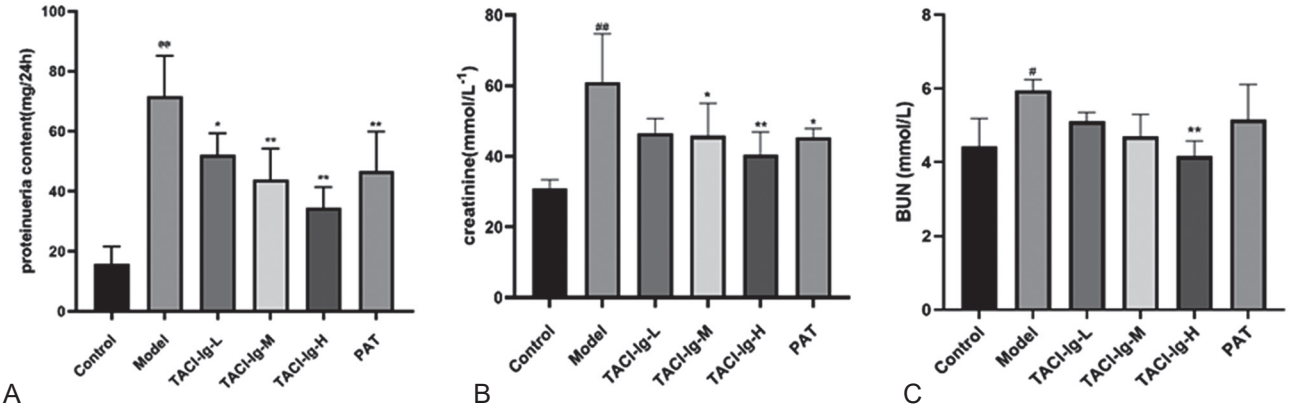

Figure 2. Effects of TACI Ig on 24 h UP, urea, and blood creatinine in IgAN rats. (A) 24 h UP (B) blood creatinine (C) urea nitrogen; Compared with the normal group, #*P* < .05, # #*P* < .01; Compared with the model group, **P* < .05, * **P* < .01. Each group of samples has *n* = 5.

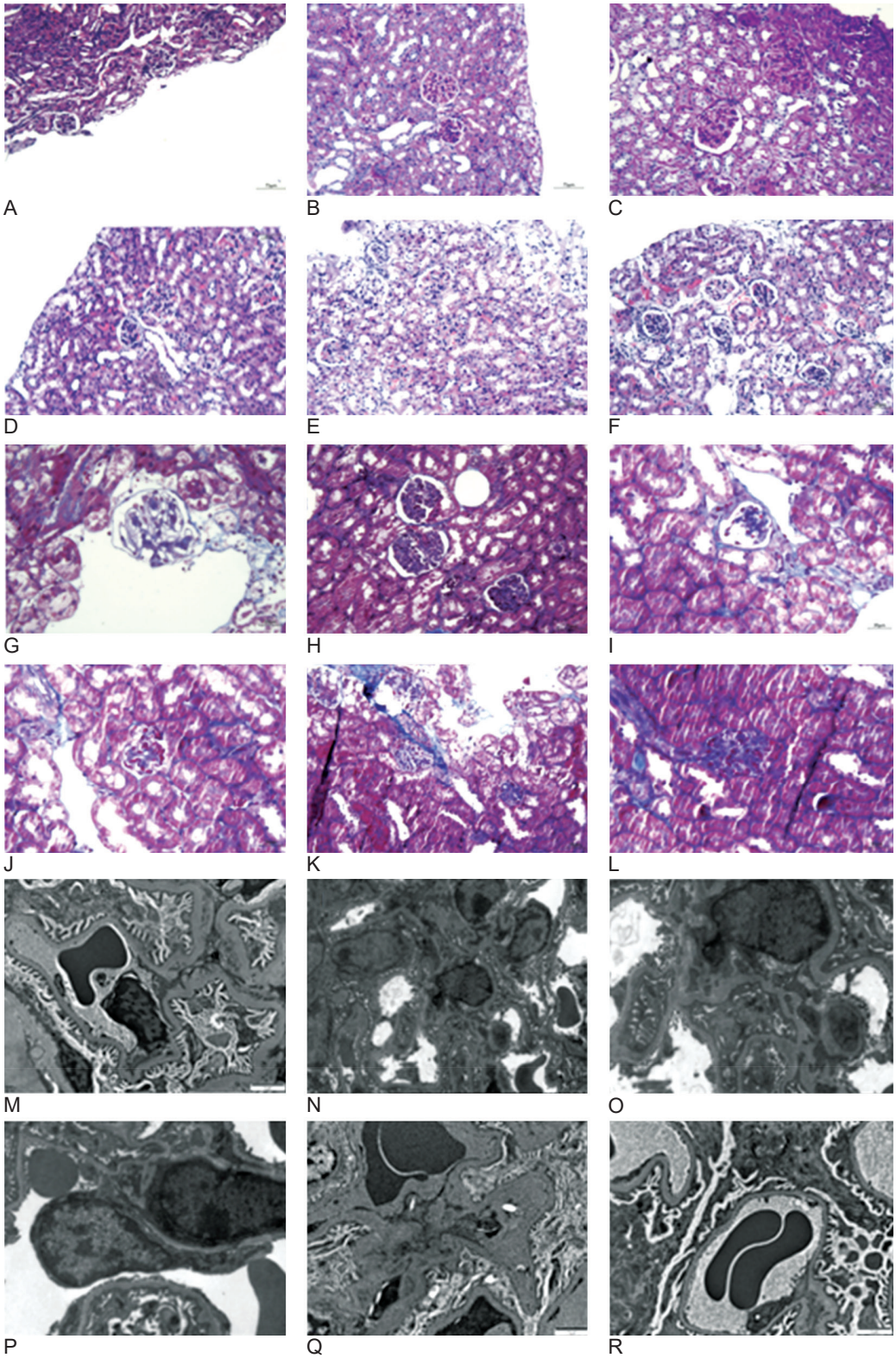

Figure 3. HE and PAS staining and Transmission electron microscopy results of renal tissue. HE staining (A-F): (A) normal control group; (B) IgAN model group; (C) TACI-Ig low-dose group; (D) TACI-Ig medium dose group (E) TACI-Ig high-dose group; (F) Drug control group. PAS staining (G-L): (G) normal control group; (H) IgAN model group; (I) TACI-Ig low-dose group; (J) TACI-Ig medium dose group (K) TACI-Ig high-dose group; (L) Drug control group; Transmission electron microscopy (M-R): (M) normal control group; (N) IgAN model group; (O) TACI-Ig low-dose group; (P) TACI-Ig medium dose group (Q) TACI-Ig high-dose group; (R) Drug control group.

model group rats were higher than those in control group (Figure 4A and B, BLyS: 1.003 pg/mL *vs* 2.311 pg/mL, APRIL: 7.766 pg/mL *vs* 21.11 pg/mL, $P < .05$), significantly reduced after treatment with high dose TACI-Ig (Figure 4A and B, BLyS: 1.254 pg/mL *vs* 2.311 pg/mL, APRIL: 16.49 pg/mL *vs* 21.11 pg/mL, $P < .05$). The Gd-IgA1 in IgAN model group was higher than normal control group (Figure 4C), indicating that the IgAN rats were in an immune damaged state. After TACI-Ig treatment, the Gd-IgA1 expression in each group decreased to varying degrees. TACI-Ig reduced the degree of immune damage in the TACI-Ig treatment group rats.

The effect of TACI fusion protein on the deposition of fluorescent IgA

Immunofluorescence analysis shows that TACI-Ig effectively decreases the deposition of IgA in renal tissue, particularly in medium and high dose groups of TACI-Ig (Figure 5D-E). TACI-Ig can alleviate renal immune damage by significantly

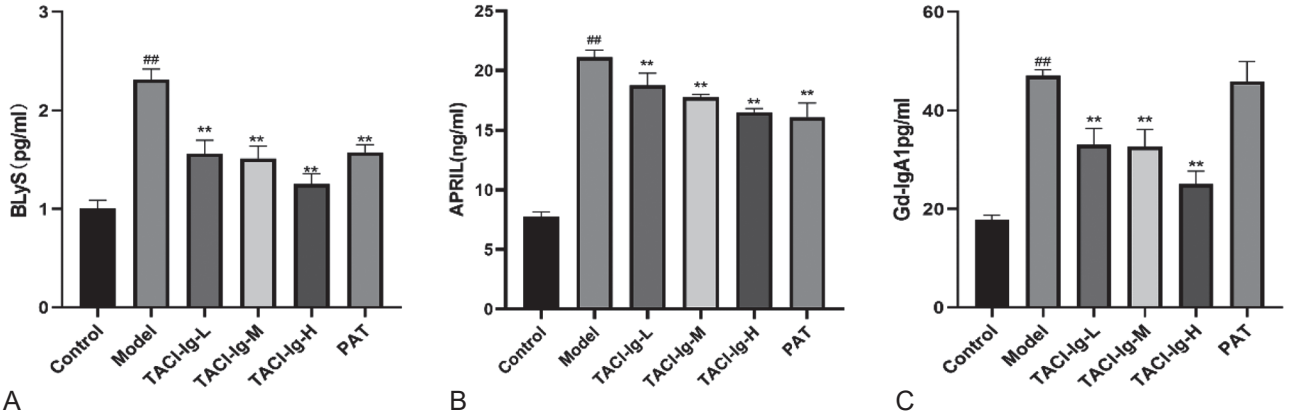

Figure 4. TACI-Ig affects the expression of BlyS, APRIL, and Gd-IgA1 in serum of IgAN rats. (A) BlyS (B) APRIL (C) Gd IgA1. Compared with the normal group, #$P < .05$, # #$P < .01$; Compared with the model group, *$P < .05$, * *$P < .01$. Each group of samples has $n = 5$.

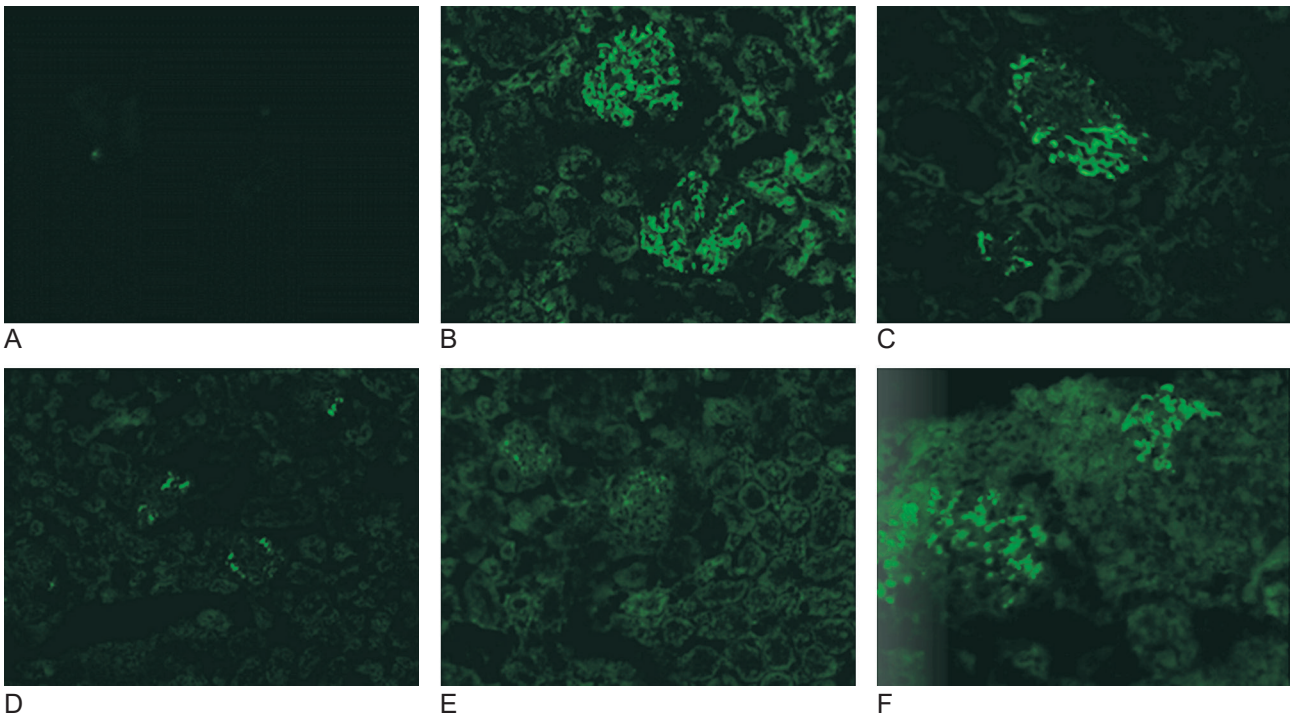

Figure 5. Immunofluorescence staining results of renal tissue (A) in the normal control group; (B) IgAN model group; (C) TACI-Ig low dose group; (D) TACI-Ig medium dose group (E) TACI-Ig high dose group; (F) Drug control group.

inhibiting the deposition of immune complexes.

The influence of TACI fusion protein onTLR4/MyD88/NF-κB signal *in vivo*

As shown in Figure 6A, the level of TLR4, MyD88, and NF-κB protein in model groups were increased, and reduced in TACI-Ig high dose group (Figure 6B-D). The model group *vs* control group had TLR4: 0.369 ± 0.077 *vs* 0.986 ± 0.223, MyD88: 0.321 ± 0.099 *vs* 0.877 ± 0.118, NF-κB: 0.393 ± 0.077 *vs* 1.070 ± 0.249, all $P < .01$). The ratio of TACI-Ig high dose group to IgAN model group, TLR4: 0.501 ± 0.080 *vs* 0.986 ± 0.223, MyD88: 0.439 ± 0.185 *vs* 0.877 ± 0.118, NF-κB: 0.563 ± 0.105 *vs* 1.070 ± 0.249 ($P < .05$).

TLR4, MyD88, and NF-κB mRNA level expression in renal tissue

As shown in Figure 7A-C, the mRNA level of TLR4, MyD88, NF-κB in renal tissue of IgAN model group was higher than normal control group. TACI-Ig could reduce the RNA expression of TLR4, MyD88, NF-κB in renal tissue ($^{\#\#}P < .01$). After 8 weeks of TACI-Ig treatment in IgAN rats, the mRNA expression of TLR4, MyD88, NF-κB detected in renal tissues of different groups were lower to varying degrees than those of IgAN model group ($^{*}P < .05$).

DISCUSSION

As a glomerular disease, the clinical symptoms of IgAN can progress from asymptomatic proteinuria to hematuria in a short period of time.[17,18] Studies have found that urinary Gd IgA1 levels are associated with immune response, inflammatory response and intestinal barrier dysfunction, which are also pathological characteristics of IgAN. Therefore, urine Gd IgA1 may be of great reference

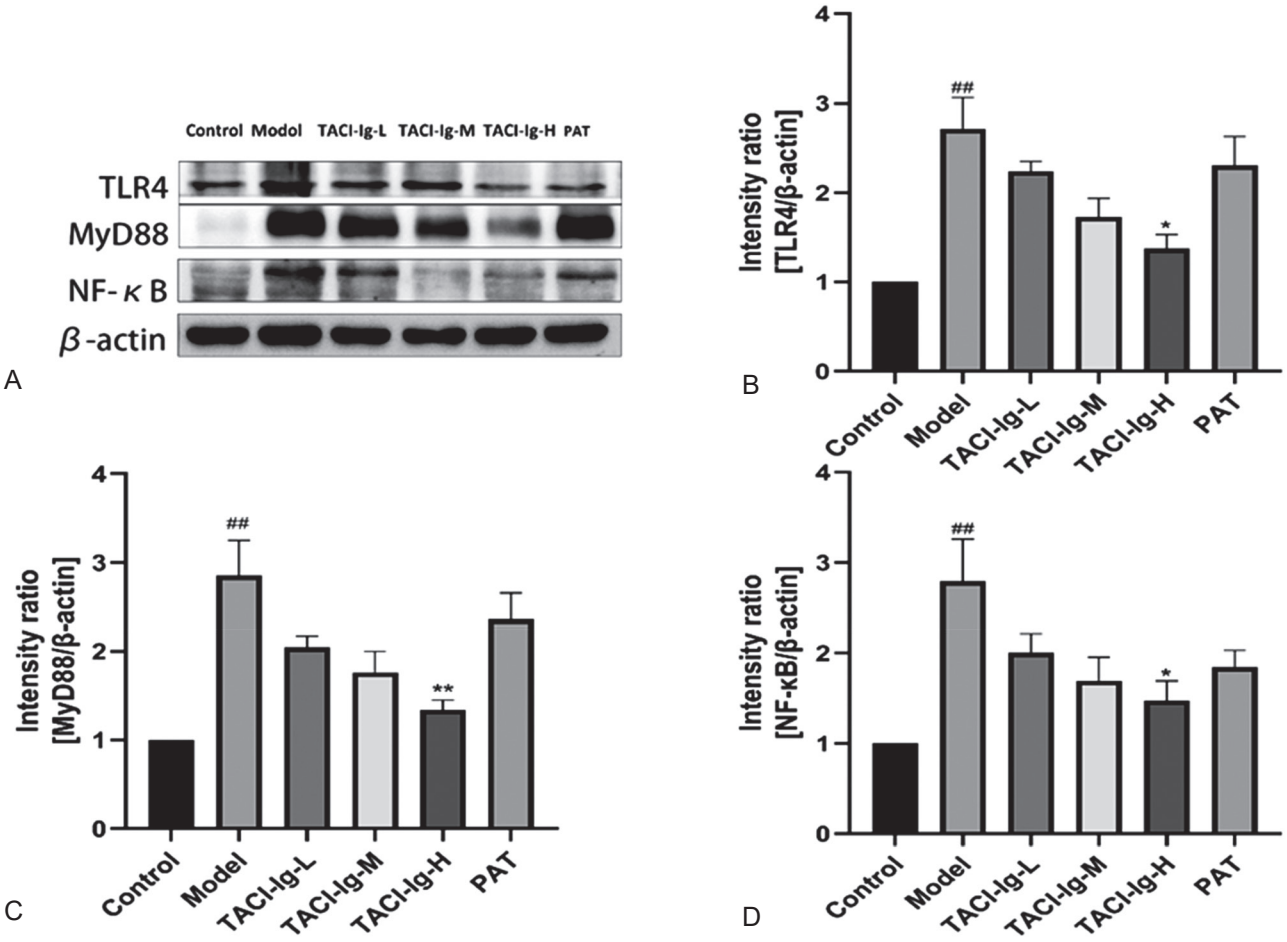

Figure 6. TLR4/MyD88/NF-κB in renal tissue protein expression results; Figure 6A showed the original gel electrophoresis, and Figure 6B-D showed the expression rate compared with NC group. Compared with the normal group, $\#P < .05$, $\#\#P < .01$; Compared with the model group, $^{*}P < .05$, $^{**}P < .01$. Each group of samples has n = 5.

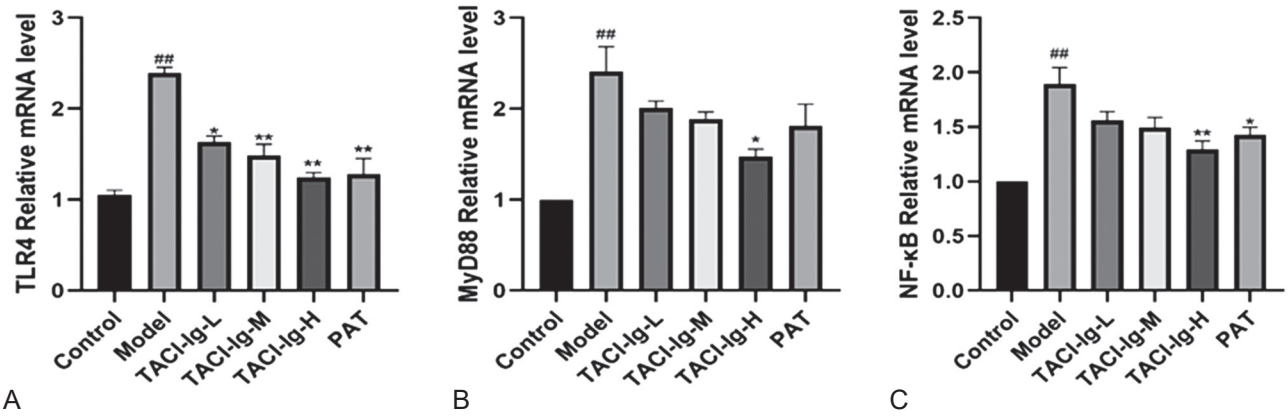

Figure 7. TLR4, MyD88, and NF-κB mRNA expression result in renal tissue (A) TLR4; (B) MyD88; (C) NF-κB. Compared with the normal group, #*P* < .05, # #*P* < .01; Compared with the model group, **P* < .05, * **P* < .01. Each group of samples has n = 5.

value in the early screening of IgAN.[19]

Abnormal changes in serum BLyS levels were detected in a variety of autoimmunity.[20] Both BLyS and APRIL play an important role in the development of B lymphocytes, and abnormal changes and expressions of BLyS and APRIL appear in serum and target organs of patients with autoimmune diseases.[4,21] APRIL is involved in the pathogenesis of IgAN and mediates the excessive production of pathological Gd-IgA1.[22] APRIL has an important association with the pathogenesis of IgAN, and its change is the cause of the massive secretion of pathological Gd-IgA1.[23] In this study, through the construction of IgAN model group of rats, it was found that the serum BAFF and APRIL levels of rats in the model group were significantly higher than those of the control group, indicating that the kidney tissue of rats in the model group was damaged.

TACI fusion protein is recombinant fusion protein with a ligand binding domain of TACI receptor and Fc component of human IgG. It is a dual inhibitor of BAFF/APRIL. [25] Domestic Phase II clinical trial data showed that the urinary protein levels of subjects significantly decreased compared to baseline.[24] In addition, compared to baseline, the average 24 h UP level decreased by 49%, indicating that TACI Ig can play a role in IgAN.

In this study, we demonstrated that NF-κB signal in the IgAN rat model was activated, which suggested that BAFF/APRIL may be involved in the pathogenesis of IgAN in rats. TACI-Ig can significantly reduce the production of proteinuria, BUN, and SCr, as well as the deposition of immune complexes and serum Gd-IgA1 levels. Its mechanism may be related to the inhibition of TLR/NF-κB signaling pathway which is related to reducing inflammatory response. However, the mechanism by which TACI-Ig protects IgAN is not clear. Next, we investigated TLR/NF-κB signaling pathway and found that TACI-Ig inhibited the activation of TLR/NF-κB pathway, thereby alleviating kidney damage.

TLR plays an important role in the body's immune function. It regulates the body's immunity through the recognition of pathogens, and TLR also plays a key role in the processes of inflammation, infection and allergy.[8] TLR4-mediated LPS, binding to intestinal mucosal epithelial cells, can cause direct damage to intestinal mucosal function and trigger B cell activation, resulting in the production of low-affinity IgA and ultimately leading to the development of IgAN.[9] When TLR4 activity is triggered, nuclear factor kappa-B (NF-κB) is activated through the MyD88 dependent pathway. [26] Through analysis of infertile IgAN mice, studies have found that intestinal flora imbalance will damage the intestinal mucosal barrier of the body, forming adverse stimulation and activating TLR4 pathway, mediating IgAN kidney injury.[27] This pathway also produces Gd-IgA1, and interacts with IgG, IgM, complement C3 and other substances to affect kidney tissue and increase the risk of nephritis.[28] NF-κB is an important transcription factor, which has a significant effect on inflammatory response and the release of related inflammatory mediators, and its expression level may greatly affect the pathogenesis of chronic kidney disease. Therefore, TLR/NF-κB may be an important regulatory factor of IgAN.

CONCLUSION

Results of this study indicates that TACI-Ig has

notable therapeutic effect on IgAN, improves renal function in rats with glomerulonephritis, inhibits the expansion of glomerular mesangial matrix and proliferation of mesangial cells. In addition, it was found that TLR4/MyD88NF-κB pathway is involved in the pathogenesis of IgAN rats, and its activation ultimately leads to glomerular injury. However, TACI-Ig can inhibit the activation of TLR4/MyD88NF-κB pathway. In summary, TACI-Ig showed a promising therapeutic potential as a novel therapeutic drug in this study, while the TLR4/MyD88/NF-κB pathway was down-regulated and the expression of BlyS and APRIL, thereby improving glomerular function and structural damage in rats with IgAN, which may be one of the therapeutic mechanisms for IgAN.

Limitations

It is necessary to acknowledge the limitations of this work. Because TACI-Ig is relatively new and there are few previous studies on IgAN, the lack of experience may cause bias in the results. TACI-Ig shows the side effects of skin allergy on the site of local injection. TACI-Ig can effectively inhibit BlyS, but whether the additional receptors of BlyS and APRIL are directly affected by the drug has not been accurately concluded, and further studies will continue in the future.

ACKNOWLEDGEMENT

None.

CONFLICT OF INTERESTS

Authors declare no conflict of interests.

CONSENT FOR PUBLICATIONS

All the authors read and approved the final manuscript for publication.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The experimental plan was approved by the Medical Ethics Committee of the First Affiliated Hospital of Baotou Medical College, Inner Mongolia University of Science and Technology (2023 Ethics Review (clin) No. (014)).

INFORMED CONSENT

The authors declare that no patients were used in this study.

AVAILABILITY OF DATA AND MATERIAL

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AUTHORS' CONTRIBUTIONS

Jianhua Sun, Yue Cheng and Zengyan Li designed the study and performed the experiments, Wei Zhang and Peng Chen collected the data, Xinnan Chen and Caili Wang analyzed the data, Jianhua Sun, Yue Cheng and Zengyan Li prepared the manuscript. All authors read and approved the final manuscript.

FUNDING

This study was supported by the Projects of Health Science and Technology Funds of baotou (grant no. 2024wsjkkj62).

REFERENCES

1. Schena FP. A retrospective analysis of the natural history of primary IgA nephropathy worldwide. Am J Med. 1990; 89(2): 209-215.
2. Berthoux FC, Mohey H, Afiani A. Natural history of primary IgA nephropathy. Semin Nephrol. 2008; 28(1): 4-9.
3. Cheung CK, Barratt J. The role of BAFF and APRIL in IgA nephropathy: pathogenic mechanisms and targeted therapies. Front Nephrol. 2024;1;3:1346769.
4. Moisini I, Davidson A. BAFF: a local and systemic target in autoimmune diseases. Clin Exp Immunol. 2009; 158(2): 155-163.
5. McAllister E, Jellusova J. BAFF signaling in B cell metabolism. Curr Opin Immunol. 2021; 71: 69-74.
6. Shabgah AG, Shariati-Sarabi Z, Tavakkol-Afshari J, et al. The role of BAFF and APRIL in rheumatoid arthritis. J Cell Physiol. 2019; 234(10): 17050-17063.
7. Wong DP, Roy NK, Zhang K, et al. A BAFF ligand-based CAR-T cell targeting three receptors and multiple B cell cancers. Nat Commun. 2022; 13(1): 217.
8. Kalled SL. The role of BAFF in immune function and implications for autoimmunity. Immunol Rev. 2005; 204: 43-54.
9. Zhang H, Sun SC. NF-kappaB in inflammation and renal diseases. Cell Biosci. 2015; 5: 63.
10. Dechkajorn W, Benjathummarak S, Kumsiri R, et al. The role of the BAFF/APRIL system in the T cell-independent specific response to blood stage Plasmodium falciparum hemozoin. Cytokine. 2018; 111: 445-453.
11. Yeo SC, Cheung CK, Barratt J. New insights into the pathogenesis of IgA nephropathy. Pediatr Nephrol. 2018; 33(5): 763-777.
12. Lee M, Suzuki H, Ogiwara K, et al. The nucleotide-sensing Toll-Like Receptor 9/Toll-Like Receptor 7 system is a potential therapeutic target for IgA nephropathy. Kidney

Int. 2023; 104(5): 943-955.

13. He H, Shen M, Tang Y, et al. LPS/TLR4 Pathway Regulates IgA1 Secretion to Induce IgA Nephropathy. Altern Ther Health M. 2024; 30(1): 419-425.
14. Dhillon S. Telitacicept: First Approval. Drugs. 2021; 81(14): 1671-1675.
15. Shi F, Xue R, Zhou X, et al. Telitacicept as a BLyS/APRIL dual inhibitor for autoimmune disease. Immunopharm Immunot. 2021; 43(6): 666-673.
16. Zhang L, Jin H, Wang D, et al. Case report: Successful treatment of refractory membranous nephropathy with telitacicept. Front Immunol. 2023; 14: 1268929.
17. Barratt J, Lafayette RA, Zhang H, et al. IgA nephropathy: the lectin pathway and implications for targeted therapy. Kidney Int. 2023; 104(2): 254-264.
18. Pattrapornpisut P, Avila-Casado C, Reich HN. IgA Nephropathy: Core Curriculum 2021. Am J Kidney Dis. 2021; 78(3): 429-441.
19. Tang Y, Xiao Y, He H, et al. Aberrant Gut Microbiome Contributes to Barrier Dysfunction, Inflammation, and Local Immune Responses in IgA Nephropathy. Kidney Blood Press R. 2023; 48(1): 261-276.
20. Gross JA, Dillon SR, Mudri S, et al. TACI-Ig neutralizes molecules critical for B cell development and autoimmune disease. impaired B cell maturation in mice lacking BLyS. Immunity. 2001; 15(2): 289-302.
21. Prieto-Pena D, Genre F, Remuzgo-Martinez S, et al. BAFF, APRIL and BAFFR on the pathogenesis of Immunoglobulin-A vasculitis. Sci Rep-Uk. 2021; 11(1): 11510.
22. Yeo SC, Barratt J. The contribution of a proliferation-inducing ligand (APRIL) and other TNF superfamily members in pathogenesis and progression of IgA nephropathy. Clin Kidney J. 2023; 16(Suppl 2): ii9-ii18.
23. Mockel T, Basta F, Weinmann-Menke J, et al. B cell activating factor (BAFF): Structure, functions, autoimmunity and clinical implications in Systemic Lupus Erythematosus (SLE). Autoimmun Rev. 2021; 20(2): 102736.
24. Mathur M, Barratt J, Chacko B, et al. A Phase 2 Trial of Sibeprenlimab in Patients with IgA Nephropathy. New Engl J Med. 2024; 390(1): 20-31.
25. Cai J, Gao D, Liu D, et al. Telitacicept for autoimmune nephropathy. Front Immunol. 2023; 14: 1169084.
26. Abdel-Aziz AM, Fathy EM, et al.TLR4/MyD88/NF-κB signaling pathway involved in the protective effect of diacerein against lung fibrosis in rats. Hum Exp Toxicol. 2023; 42:9603271231200213.
27. Tang Y, Sun W, He H, et al. Activation of intestinal mucosal TLR4/NF-kappaB pathway is associated with renal damage in mice with pseudo-sterile IgA nephropathy. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi. 2023; 39(10): 865-871.
28. Nihei Y, Suzuki H, Suzuki Y. Current understanding of IgA antibodies in the pathogenesis of IgA nephropathy. Front Immunol. 2023; 14: 1165394.

*Correspondence to:
Zengyan Li, MD
Department of Nephrology, The First Affiliated Hospital of Baotou Medical College, Inner Mongolia University of Science and Technology, No.41, Linyin Street, Kun District, Baotou 014040, China.
Email: lizengyan572@aliyun.com

Revised March 2024
Accepted May 2024