# Immunization-induced Establishment and Serum Expression of BAFF/Gd-IgA1 in IgAN Rats

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***Abstract:*** *The rat model of IgA nephropathy induced by immunogen plus endotoxin was established, and the drug dose and method improved on the basis of IgA nephropathy model were carried out to establish a more suitable rat model of clinical IgA nephropathy. BAFF and Gd-IgA 1 expression were measured from the serum and kidney tissues of IgA nephropathy rats. SD males, 24 mice with 0.18-0.2Kg body mass, were randomly divided into control and model groups. Model: immunized bovine serum albumin (Bovine Serum Albumin V, BSA) dissolved at 150g / L, 600mg / Kg the next day, premixed with castor oil and carbon tetrachloride, concentration 3:1; subcutaneous 0.4ml / only once a week for 12 weeks. A 0.05mg dose of lipopolysaccharide (Lipopolysaccharides, LPS) was administered in the tail vein at weeks 6,8, and 10, and the control group in saline. At the end of week 12 of intervention, blood was collected from the abdominal aorta for 24-h proteinuria (24h pro), serum creatinine (SCr) and urea nitrogen (BUN). HE, PAS pathological staining, electron microscopy and light microscopy, and BLyS and Gd-IgA 1 expression in serum and kidney tissues using ELISA experiments. In the model group, the content of proteinuria, Cre and BUN increased significantly at 24h, and the pathological staining results showed obvious morphological changes, and the area of enhanced fluorescence positive by pathological immunofluorescence staining increased. BLyS and Gd-IgA 1 expression were higher in serum and kidney tissues than in the blank control group. The modified molding method of IgA nephropathy had good results, and the pathological and clinical indicators were close to clinical IgA nephropathy.*

***Keywords:*** *Immunization, BAFF/Gd-IgA1, IgA Nephropathy (IgAN)*

## 1. Introduction

IgA nephropathy (IgAN) was first proposed by Berger and Hinglais and is considered the most common[1] of primary glomerulonephritis. IgA nephropathy has different heterogeneity in terms of clinical presentation and risk of progression, with early symptoms being microscopic hematuria[2-3], and is the leading cause of end-stage renal disease worldwide[4]. The main histological features of IgAN are IgA granular mesangial immunoprecipitation associated with mesangial cell proliferation and matrix expansion[5], multiple hit hypothesis, including galactose-deficient IgA 1, IgG or IgA autoantibodies, and their subsequent immune complex formation and glomerular deposition, have been widely supported by many studies[6]. To develop effective treatments for IgAN, appropriate animals are urgently needed to elucidate the pathogenesis of IgAN[7]. The earliest IgA nephropathy model is a spontaneous model of ddY mice, but the main disadvantages are the age of onset and the high variation of the disease. In China, bovine serum albumin (BSA) + carbon tetrachloride (ccl 4) + lipopolysaccharide (LPS) is mostly used to establish IgAN rat model[8]. This method is convenient and simple, repetitive, high success rate and is widely used in China. Previous studies have successfully reproduced rat IgAN using this method, but the molding effect is not ideal due to the low dose of BSA. Therefore, this experiment improved the method of the IgAN rat model and observed the effect.

## 2. Materials

Animals: 208-week-old male SPF SD rats with a body weight of 180-200g were purchased from Peking University Health Science Center (Laboratory Animal Science Department). Animal License No.: SCXK (Jing) 2021-0013. Animals were maintained at the Experimental Animal Center of Baotou Medical College.

Reagent: bovine serum albumin (BSA, A8020) was purchased from Beijing; lipopolysaccharide (LPS, L8880) was purchased from Beijing; carbon tetrachloride (CCl4, C805332), castor oil (C805202) was purchased from Shanghai; and rabbit anti-rat IgA antibody (bs-10491R) was purchased from Beijing.

## 3. Experimental methods

### 3.1 Group grouping, model establishment and disposal method

Twenty-four SPF SD rats were kept for 1 week and divided into three groups according to the random number table method, control group (n=10), model A (n=10), and Model B (n=10). According to the IgA nephropathy modeling method of Sun Hongxu[9], and improve it on the basis. Model Group A: BSA pure water solution of 150g / L on the first day of the experiment, drug dose of 600mg / Kg for 9 weeks; premixed castor oil and carbon tetracloride, concentration 3:1; subcutaneous 0.4ml / only once a week for 9 weeks; lipopolysaccharide (LPS) weeks 6 and 80.05mg tail vein. Model group B: BSA pure water was filled with the above concentration solution, and the infusion time was extended to 12 weeks; 0.05mg in tail vein at weeks 6,8 and 10 for LPS injection; the control group used normal feeding with saline for 12 weeks. After blood collection, both kidneys were harvested, and the renal cortex was wrapped with saline soaked gauze and sealed for cold storage. IgA immunofluorescence was performed, and some paraformaldehyde was placed for light microscopy.

### 3.2 24-hour urine protein and renal function measurement in rats with IgA nephropathy

At the end of the 10th and 12th, the rats were placed in the rat metabolic cage, and the 24-hour urine volume was collected and recorded, and the 24-hour urine protein quantification was detected by automatic biochemical analyzer. After the end of the intervention, blood was collected from the abdominal aorta, and 30min later, the supernatant was removed by centrifugation at 3000 r/min-1 for 15min, and serum SCr and blood BUN levels were measured by an automatic biochemical analyzer.

### 3.3 Renal histological examination

### 3.3.1 Light examination

The kidney tissues were fixed in paraformaldehyde for 24 hours, embedded in paraffin, and stained with hematoxylin-eosin and acid acid. The proliferation of cells and matrix in the mesangial region of glomerulus were observed by optical microscopy.

### 3.3.2 Immunofluorescence staining

Kidney tissue embedded section, fixed for 10min, PBS rinsing 3 times, drop with rabbit anti-rat IgA antibody (1:50), 4 degrees cassette overnight, PBS washing 3 times, FITC pig anti-rabbit fluorescent secondary antibody labeling, 37℃ cassette incubated for 30min, PBS washing 3 times, fluorescence confocal microscope observation, glomerular IgA deposition characteristics.

### 3.4 The BLyS and Gd-IgA 1 levels were determined by ELISAELISA

The kit strictly follows the instruction procedure.

### 3.5 Statistical methods

Statistical analysis was performed using the SPSS 20.0 software. The measurement data are represented by ($x \pm s$), and singles are compared between multiple groups Analysis of factor variance (ANOVA) analysis, and rank sum test was used for rank measurement data. $P < 0.05$ indicates statistical significance Sense, $P < 0.01$ was considered as being statistically significant.

## 4. Results

### 4.1 General situation

During the experimental molding process, during the subcutaneous injection of CCL 4 in model A and B, the rats struggled to varying degrees, screamed loudly, and blew up their hair. After subcutaneous

injection, they were depressed, moved slowly, and neither drank or ate. After tail vein injection of LPS, depression and fecal incontinence appeared, which resolved after 1 day. The control group was in good mental condition, with normal weight increase, and no abnormalities in eating and defecation. There were no deaths in the control group and the models in groups A and B.

### 4.2. The 24h urine protein quantification and comparison of SCr and BUN

After 12 weeks, 24h urine protein quantitative model B rats were significantly higher than the control group (P <0.01). Model group A and control group compared with group B was statistically significant. In terms of renal function, the serum creatinine of model A and model B rats improved compared with the control group, and the serum creatinine of model B and control group.(All P <0.05) At the end of 12, model A and Model B compared with the control group (all P <0.05). The results are shown in Table 1.

*Table 1  24-hour urinary protein and changes in renal function in rats*

| group | n | 24h Pro(mg·24h$^{-1}$) | SCr(μmol·L$^{-1}$) | BUN(mmol·L$^{-1}$) |
|---|---|---|---|---|
| control group matched group | 8 | 2.14±0.34 | 29±3.12 | 3.36±0.41 |
| Model Group A | 8 | 5.86±0.64## | 37.75±3.30# | 4.33±0.65## |
| Model Group B | 8 | 10.62±1.08##** | 48.88±10.02##** | 6.46±0.55##** |

VS normal control group # p (0.05, # # p (0.01; VS model group A, * p (0.05)

### 4.3 Light microscopy for observation

The glomeruli of the rats in the control group showed normal morphology and structure, and the mesangial cells and matrix did not show abnormal proliferation and fibrosis. In group A, only the tubular injury and no severe hyperplasia of glomeruli and stroma. In model group B, the glomerular balloon space was slightly enlarged, with diffuse hyperplasia of mesangial cells and mesangial stroma, and a few glomeruli may have segment hyperplasia, which was similar to clinical IgAN, Figure 1.
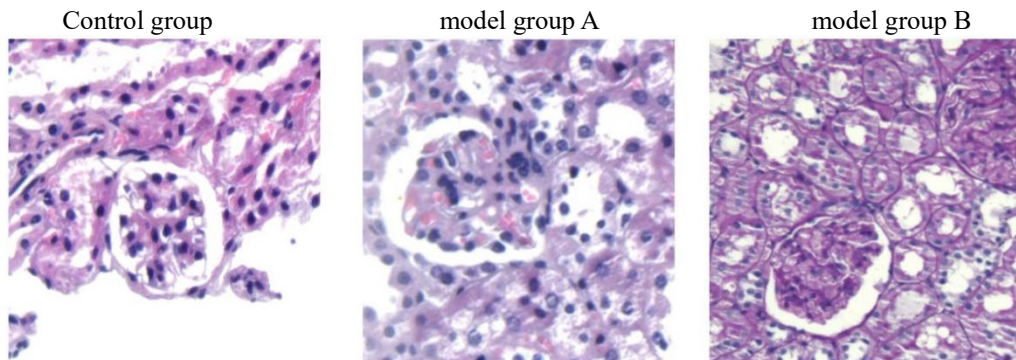
Control group          model group A          model group B



*Figure 1. Pathological HE staining of rat kidneys in each group*

### 4.4. Immunofluorescence observation

### 4.4.1 Deposit strength of IgA

The results of immunofluorescence in group were: no obvious fluorescence occurred in control rats, but no fluorescence deposition was observed in glomeruli; in model group B, fluorescence deposition occurred in tubules, and in the mesangial area, as shown in Figure 2.
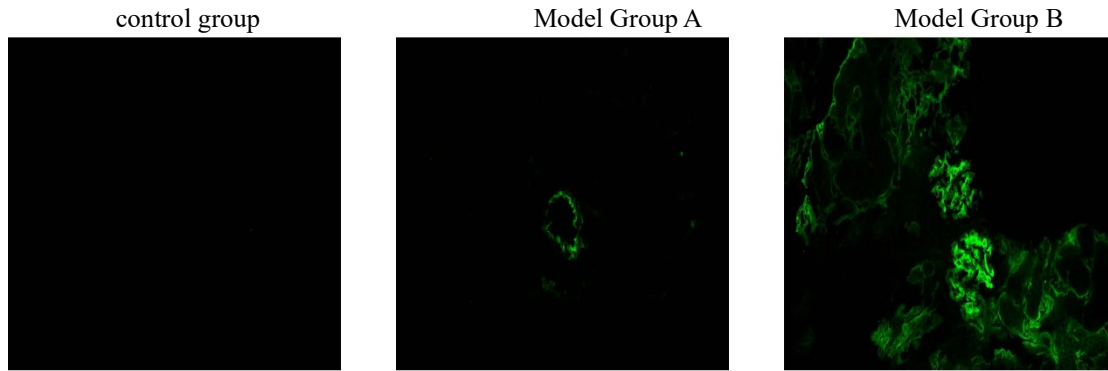
| control group | Model Group A | Model Group B |
| --- | --- | --- |



*Figure 2. Immunofluorescence precipitation of rats in each group*

### 4.4.2 IgA Deposition strength score

Model Group A was positive (+ ~ + +) under immunofluorescence and model Group B (+ + ~ + + + +). The control group was presented as a (- ~ +) group. The model and control groups were analyzed using the non-parametric rank and sum test H=17.585  P=0.000 (see Table 2).

*Table 2. Immunofluorescence intensity scores of kidney tissues in each group*

| group | n | – | + | ++ | +++ | ++++. |
| --- | --- | --- | --- | --- | --- | --- |
| control group; matched group | 8 | 5 | 3 | | | |
| Model Group A | 8 | | 3 | 5 | | |
| Model Group B | 8 | | | 4 | 3 | 1 |

### 4.5 Serum levels of BLyS and Gd-IgA 1 were determined

To further evaluate the IgAN rat model, we determined the expression of galactose-deficient antibody (Gd-IgA 1) and B lymphocyte-stimulating factor (BAFF) in IgAN rat serum using ELISA. Gd-IgA 1 was significantly higher in the serum of model rats than in control rats, compared with model A, and decreased BAFF and Gd-IgA 1, as shown in Table 3.

*Table 3. Serum levels of BAFF and Gd-IgA 1 expression in rats of each group*

| group | n | BLyS (pg/ml) | Gd- IgA1 (pg/ml) |
| --- | --- | --- | --- |
| control group; matched group | 8 | 0.92±0.13 | 5.12±0.91 |
| Model Group A | 8 | 1.28±0.12[#] | 39.05±6.10[#] |
| Model Group B | 8 | 1.61±0.15[#*] | 47.15±2.66[#*] |

Comparison with normal group, # P (0.05±1.2); compared to model group, * P (0.05±2.05).

## 5. Discuss

IgA nephropathy (IgAN) is the most common glomerulonephritis worldwide and the leading cause of chronic kidney disease and renal failure. Diagnosis of IgA nephropathy requires renal biopsy, at least by light microscope and fluorescence microscopy, and immunofluorescence microscopy is a necessary [10] for the identification of IgA deposition. The pathogenesis of IgAN in IgA nephropathy is relatively complex, and there is no specific treatment plan[11]. In order to elucidate the pathogenesis and treatment methods of IgAN, appropriate animals are urgently needed to contribute to the change of IgAN treatment. There was no death in the molding process of this experiment, and the success rate of mold making reached more than 90%. Compared with model group A, the 24-hour urinary protein, renal function, renal histopathological staining and immunofluorescent deposition were more significant, and the symptoms of the experimental immune-induced IgA nephropathy model were more consistent with those of clinical IgA nephropathy. Moreover, the expression of serum BAFF and Gd-IgA 1 in model group B rats further proved that the method of establishing model group B rats was better than that of model group A.

## 6. Conclusion

In conclusion, this experiment concluded that BSA (600 mg Kg-1) was gavage with purified water, weekly subcutaneous injection of CCl 4 for 12 weeks, tail vein injection of LPS at weeks 6,8, and 10, IgA nephropathy was more typical and prominent. From the results of immunofluorescence, the intensity of immunofluorescence as well as the deposition of immune complexes in the mesangial zone was increased by extending the modeling time and increasing the tail vein injection. This experiment combines the experience of domestic scholars in establishing IgA animal model. On the other hand, it also suggests that the operation time can be extended and increased by intraperitoneal injection of CCl4, and a more stable and high fluorescence intensity animal model of IgA nephropathy can be obtained. Due to the batch differences in experimental animals, individual differences, and varying sensitivities to drugs, the development of IgA nephropathy is a dynamic process. During the modeling process, to better observe the evolution of pathology, it is necessary to detect changes in renal function, pathology, and immunofluorescence in the animal model. To minimize harm to the animals, it is important to explore the optimal time limit for modeling as soon as possible.

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