ORIGINAL RESEARCH

LPS/TLR4 Pathway Regulates IgA1 Secretion to Induce IgA Nephropathy

Haidong He, PhD; Meilin Shen, BS; Yuyan Tang, MM; Weiqian Sun, MM; Xudong Xu, MM

ABSTRACT

Context • Studies have reported that the incidence and severity of IgA nephropathy (IgAN) are closely related to the imbalance of the intestinal flora. Imbalance of the intestinal flora may cause abnormalities, such as intestinal mucosal immunity or mesenteric B1 lymphocyte subsets. These can lead to an increase in immunoglobulin A (IgA) production and IgA structural changing, which can eventually cause IgA1 deposition in the glomerular mesangial area and nephritis.

Objective • The study intended to explore whether the LPS/TLR4 pathway regulates mesenteric B cells, secreting Gd-IgA1 to induce IgA nephropathy.

Design • The research team designed an animal study.

Setting • The study took place at Department of Nephrology, Minhang Hospital, Fudan University.

Animals • The animals were 60 specific pathogen free (SPF) C57BL/6 (B6, H-2b) male mice from that were 6-8 weeks old and weighed 20-25 grams.

Intervention • The research team established a mouse model of IgA nephropathy. The team created five groups of mice: (1) the NC group, a normal negative control group without induced nephropathy and with no treatments; (2) the IgA nephropathy (IgAN) group, a positive control group with induced nephropathy and with no treatments; (3) the IgAN+anti-TLR4 group, an intervention group, with induced nephropathy and with a TLR4-antibody (anti-TLR4) treatment; (4) the IgAN+GEC group, an intervention group, with induced nephropathy and with treatment with glutamine enteric-coated capsules (GEC); and (5) the IgAN+anti-TLR4+GEC group, an intervention group, with induced nephropathy and with treatment with anti-TLR4 and GEC.

Outcome Measures • The research team collected the blood and urine of all the mice and used an enzyme-linked immunoassay (ELISA) to analyze the levels of blood creatinine, urine protein, and urea nitrogen (BUN). The team also used the ELISA to analyze signal molecules for serum inflammation: interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), monocyte chemotactic protein 1 (MCP-1), cyclooxygenase-2 (COX2), and galactose-deficient IgA1(Gd-IgA1). The team analyzed the distribution and content of IgA+B220+B lymphocytes in the intestinal tissues of all the mice, using tissue immunofluorescence tracking technology, and used hematoxylin-eosin (HE) staining to analyze the pathological damage in the kidney tissue. For analysis of glomerular IgA deposition, the team used a tissue immunofluorescence technique, and for detection of protein expression—toll-like receptor 4 (TLR4), B-cell activating factor (BAFF), and a proliferation-inducing ligand (APRIL)—in mesenteric lymphoid tissues, the team used western blot analysis.

Results • For the five groups of mice, the amount or degree of the physiological indicators and inflammatory factors that ELISA detected, the B lymphocytes and IgA sedimentation that immunofluorescence tracing measured, the kidney pathological that HE staining detected, and the expression of immune-related proteins that western blotting measured, all showed a common trend: IgAN group> IgAN+ glomerular endothelial cells (GEC) group> IgAN+anti-TLR4 group> IgAN+anti-TLR4+GEC group> NC group.

Conclusions • The TLR4 antibody and GEC for the treatment of the intestinal tract can regulate and repair intestinal function, so that IgAN can also be relieved at the same time. The results supported the hypothesis that a relationship exists between IgAN and the LPS/TLR4 pathway that regulates mesenteric B cells to secrete low-glycosylated poly-IgA1, which provides a new potential therapeutic plan for IgA nephritis. (*Altern Ther Health Med.* 2024;30(1):419-425).

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Immunoglobulin A (IgA) nephropathy (IgAN) is the most common, primary, chronic glomerular disease in the world.[1] IgA nephropathy, also known as Berger's disease, is a kidney disease caused by the deposition of immunoglobulin A (IgA) in the glomeruli. It is one of the most common primary glomerulonephropathies and is most common in young adults and children. The exact cause of the disease is not fully understood, but it is associated with immune system dysregulation and genetic factors. In patients with IgA nephropathy, due to an abnormal immune system, the body

produces too many Ig antibodies, which are deposited in the glomeruli and trigger an inflammatory response. This inflammatory reaction gradually leads to glomerular damage and renal failure. Some patients have no obvious symptoms, while others show proteinuria, hematuria (especially menstrual hematuria) and high blood pressure. Some patients may gradually develop symptoms of chronic kidney disease, such as edema, fatigue, and decreased urine output.The incidence and severity of IgAN are closely related to an imbalance of the intestinal flora, which can cause abnormalities, such as intestinal mucosal immunity or mesenteric B1 lymphocyte subsets. Terasaka et al found that the intestinally derived IgA complex is deposited in the glomerular mesangial cells, causing IgAN.[2] In addition, IgAN's pathological characterization includes that the deposits can occur in the capillary loops and also that it can cause nephritis.

Immune tolerance can lead to changes in the normal intestinal flora, which plays an important role in maintaining the intestinal mucosal barrier function and immune homeostasis. These changes can cause abnormal reactions to the microbiota, including increased absorption of digestive-tract antigens and bacterial toxins. This can trigger mucosa-associated lymphoid tissue (MALT) activation and subclinical intestinal inflammation.

When the intestinal flora is imbalanced, potentially pathogenic bacteria can increase and produce a variety of toxins. The toxins can cause inflammatory damage to the intestinal mucosal barrier. Kiryluk et al's study has genetically verified the correlation between IgAN and intestinal diseases.[3]

Two recent studies have shown that low-glycosylated poly IgA1, or galactose-deficient IgA1 (Gd-IgA1), can be called nephrogenic IgA.[4,5] Synthesizing abnormally glycosylated polymeric IgA1 can cause abnormal reactions to digestive-tract antigens and symbiotic microorganisms, and ultimately, cause kidney deposits to enter the circulatory system.[6,7] Two clinical studies have found a correlation between IgAN and the microbiota of excreta.[8,9]

Increased mucosal permeability increases the absorption of antigens, such as lipopolysaccharide (LPS), and mediates systemic inflammation.[10] LPS is a phospholipid that forms the outer membrane of gram-negative bacilli. Toll-like receptors (TLRs), the receptors of LPS, are highly expressed in the epithelial cells of intestinal mucosa and in mesenteric lymphocytes. When LPS directly contacts those epithelial cells, the permeability of the intestinal mucosal epithelium increases and damage can occur to the intestinal mucosal function.[11,12]

The LPS/TLR4 pathway regulates mesenteric B cells to secrete low-glycosylated poly-IgA1 and IgA nephritis, and the content of inflammatory factors and immune-related specific proteins play a key role. When B cells are activated, TLRs on the surface of the B cells can recognize pathogen-associated molecular patterns (PAMPs) that can induce additional B-cell activation to produce low-affinity, IgM and IgA single-cloned antibodies.[13]

Five inflammatory factors increase rapidly during the occurrence of IgAN, and are the main specific indicators for diagnosing IgAN: (1) interleukin-6 (IL-6), which can make B-cell precursors become B cells that produce antibodies; (2) tumor necrosis factor alpha (TNF-α), which promotes T cells to produce various inflammatory factors, thereby promoting the occurrence of inflammation; (3) monocyte chemotactic protein 1 (MCP-1), which has chemotactic activity on monocytes and activates monocytes and macrophages[14]; (4) cyclooxygenase-2 (COX-2), the activity of which is extremely low in normal tissue cells and the expression level of which can increase from 10 to 80 times the normal level in inflammatory cells when inflammation stimulates the cells, leading to inflammation and tissue damage[15]; and (5) Gd-IgA1, the overexpression of which can lead to IgA1 sedimentation in the glomerular mesangial and finally nephritis.[19] These five factors are signal molecules for serum inflammation.

When TLR4-mediated LPS contacts intestinal mucosal epithelial cells, it can directly damage intestinal mucosal function and stimulate B-cell activation to produce low-affinity IgA, and then induce IgAN to occur.[16] The B-cell activating factor (BAFF) is an important B-cell survival factor. McCarthy et al and Gong et al found that transgenic mice that were overexpressing BAFF had mesangial-deposited IgA and abnormally glycosylated, high circulating levels of polymerized IgA.[17,18] A proliferation-inducing ligand (APRIL) acts in the later stages of immune-related diseases, cooperating with BAFF together to regulate the function and survival of effector B cells.[19]

Although some scattered advances in the study of the mechanism of IgAN have occurred, on the whole, research about a specific regulatory mechanism is still relatively rare, and the pathogenesis of IgAN isn't completely clear.[20] This has led to a limitation on the therapeutic possibilities for treating IgAN. Exploring the relationship between intestinal-flora imbalance and IgAN can provide new methods and perspectives for treatment.[21,22] B-cell immunotherapy can also provide new ideas for its treatment,[23] and another potential way for treating it relates to the gut-renal axis regulated by microbiota and diet.[24]

Therefore, the current study intended to explore whether the LPS/TLR4 pathway regulates mesenteric B cells, secreting Gd-IgA1 to induce IgA nephropathy.

METHODS

Animals

The research team designed an animal study. The study took place at Department of Nephrology, Minhang Hospital, Fudan University The research team purchased 60 specific pathogen free (SPF) C57BL/6 (B6, H-2b) male mice from Shanghai Slack Laboratory Animal (Shanghai, China). All the mice were 6-8 weeks old and weighed 20-25 grams. Feeding conditions: temperature between 20°C and 24°C, relative humidity between 40% and 60%, light cycle of 12 hours of daylight and 12 hours of darkness, administration of

special diets containing nutrients such as proteins, fats, carbohydrates, vitamins and minerals.

The research protocol was reviewed and approved by the Ethical Committee and Institutional Review Board of the Minhang District Central Hospital.

Procedures

IgAN model. After one week of adaptive feeding, the research team randomly divided the mice into five groups, a control group and four groups that the team established for the IgAN mouse model using a combined method with bovine serum albumin (BSA) + staphylococcal enterotoxin B (SEB).

For the four groups in the IgAN mouse model, the research team administered by gavage 0.1% BSA acidified water, at 0.4ml/mouse, once every other day for five consecutive weeks. At the sixth week, the team injected 1% BSA buffer, at 0.4ml/mouse, into their tail veins, once a day for 3 days. At the ninth week, the team injected 0.4mg/kg SEB into their tail veins, once a week continuously 3 weeks.

Groups. The normal healthy mice in the negative control group, the NC group, received 6mmol/L and an equal amount of acidified water by gavage, and at the same time, the research team injected 0.01mmol/L of phosphate buffered saline (PBS) buffer (Shanghai Yuanye Biotechnology Co., Ltd, Shanghai, China) into their tail veins

For the four groups in the IgAN mouse model: (1) the IgA nephropathy (IgAN) group, the positive control group, received no subsequent treatments; (2) the IgAN+anti-TLR4 group received an injection in the tail vein of 216 mg/kg/d of a TLR4 antibody once a day; (3) the IgAN+GEC group received 216 mg/kg/d of glutamine enteric-coated capsules (GEC) by gavage once a day; and (4) the IgAN+anti-TLR4+GEC received an injection in the tail vein of 216 mg/kg/d of a TLR4 antibody once a day and 216 mg/kg/d of GEC injected intravenously once a day.

All the treatments for the four nephropathy groups ended at the twelfth week. All the mice were then anesthetized with isopentane. The research team collected all of their blood, urine, intestinal tissue, and kidney tissue for experimental testing.

ELISA analysis. Ten standard wells were set on an enzyme-linked immunoassay (ELISA)-coated plate (Shanghai Xuan Ya Biotechnology Co., Shanghai, China). The research team added the standard samples to the wells according to the concentration requirements and serially diluted them. The sample volume in each well was 50μl.

Then the team set a blank control well and the sample wells to be assessed. The team added no sample or enzyme-labeled reagent to the blank control well. The other steps were the same as those with the sample wells.

The research team added 40μl of sample diluent (Wuhan Eliot Bio-technology Co., Wuhan, China) to each sample well and then added 10μl of a sample, and the dilution of the sample occurred five times. The team sealed the plate with a sealing film and incubated it at 37°C for 30 minutes. After incubation, the team washed the plate with washing solution (Shanghai Yuduo Biotechnology Co., Shanghai, China) and dried it.

After drying, the team added 50μl of enzyme-labeled reagent (Shanghai Yuduo Biotechnology Co., Shanghai, China) to each well, except the blank well, and repeated the incubation and washing steps. Then the team added 50μl of developer A (Shanghai Yuduo Biotechnology Co., Shanghai, China) and 50μl of developer B (Shanghai Yuduo Biotechnology Co., Shanghai, China) in each well sequentially and developed the color at 37°C for 15 minutes in the dark, and finally, added 50μl of stop solution (Shanghai Yuduo Biotechnology Co., Shanghai, China) to stop the reaction, with the blue turning to yellow immediately. The team set the blank control well to zero and measured the absorbance (OD value) of each well in sequence at a 450nm wavelength (Shanghai Yuduo Biotechnology Co., Shanghai, China).

Tissue immunofluorescence tracer. The research team collected the intestinal and kidney tissues of the mice of each group for acid hydrolysis. After the hydrolysis, the team encapsulated the tissues with slicing fluid and made slices of each tissue using a microtome (Shanghai Yuduo Biotechnology Co., Shanghai, China).

The team placed the tissue sections in a 65°C incubator (Shanghai Yuduo Biotechnology Co., Shanghai, China) for one hour to dewax them and then used 0.5% Triton X-100 (Shanghai Yuduo Biotechnology Co., Shanghai, China) to permeate the tissue at room temperature for 10 min. The team then used citrate buffer (Shanghai Yuduo Biotechnology Co., Shanghai, China) for antigen retrieval and incubated the slices with 3% H2O2 at room temperature for 30 min to inactivate the endogenous peroxidase.

The team applied 1% BSA for blocking at room temperature for 30 min to block nonspecific epitopes, incubated the specific primary antibody (Shanghai Yuduo Biotechnology Co., Shanghai, China) according to the manufacturer's recommended instructions, and let it stand overnight in a humidified box at 4°C.

The team took out the slices the next day; rewarmed them at room temperature for 30 min; selected the corresponding, immunofluorescence, secondary antibody (Shanghai Yuduo Biotechnology Co., Shanghai, China); then incubated them at 37°C for 30 min in the dark; and under dark conditions, stained the nucleus with 4',6-diamidino-2-phenylindole (DAPI) (Shanghai Yuduo Biotechnology Co., Shanghai, China).

The team added anti-fluorescence quencher (Shanghai Yuduo Biotechnology Co., Shanghai, China) for mounting, and finally, used a fluorescence microscope (Shanghai Yuduo Biotechnology Co., Shanghai, China) to observe and take pictures.

Hematoxylin-eosin (HE) staining. The research team observed the morphological changes in the kidney tissue with HE staining under a light microscope. After fixing the kidney tissue with 4% paraformaldehyde (PFA) at 4°C overnight, the team dehydrated the tissue using gradient ethanol, permeabilized it using xylene, and embedded it with

paraffin. Following that step, the team cut the tissues into 4-μm slices and stained them with HE solution (Nanjing Jiancheng, Nanjing, Jiangsu, China) according to the manufacturer's protocols. Finally, the team mounted the slices with neutral resins and analyzed them using a light microscope (Nikon Eclipse 1000, Nikon, Tokyo, Japan).

Western blot analysis. The research team collected the mesenteric, lymphoid-tissue cells of each group and added 200 μl of cell lysate (Shanghai Yuduo Biotechnology Co., Shanghai, China) to each six-well plate. After sonication, the team lysed the cells on ice for one hour. The team centrifuged (Shanghai Yuduo Biotechnology Co., Shanghai, China) the lysed-cell sample at 12 500 g at 4°C for 15 minutes. Then, the team transferred the supernatant in the centrifuge tube to a clean centrifuge tube, used the bicinchoninic acid (BCA) protein quantification kit (Shanghai Yuduo Biotechnology Co., Shanghai, China) to quantify the protein concentration, determined the concentration of protein, and stored the samples at -80°C.

In the Western-blotting electrophoresis, the protein-loading concentration was 50 micrograms per well. After the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (Shanghai Yuduo Biotechnology Co., Shanghai, China), the team transferred and blocked the membrane. The team added the primary anti-human antibodies (Shanghai Yuduo Biotechnology Co., Shanghai, China)—TLR4, B-cell activating factor (BAFF), a proliferation-inducing ligand (APRIL),and Actin—at 1:500 dilution (Abcam, Seattle, WA, USA). Actin was the internal reference protein in the Western blot analysis.

The team incubated the samples on a 4°C shaker overnight, and after washing the samples in PBS, incubated them with secondary antibodies at 1:1000 dilution (Abcam) in a dark at room temperature for 30 minutes. Finally, the team use the developer for development and photographing.

Outcome measures. The research team collected the blood and urine of all the mice and used an enzyme-linked immunoassay (ELISA) to analyze the levels of blood creatinine, urine protein, and urea nitrogen (BUN). The team also used the ELISA to analyze signal molecules for serum inflammation: IL-6, TNF-α, MCP-1, COX2), and Gd-IgA1.

The team also analyzed the distribution and content of IgA+B220+B lymphocytes in the intestinal tissues of all the mice, using tissue immunofluorescence tracking technology. The team used hematoxylin-eosin (HE) staining to analyze the pathological damage in the kidney tissue. Changes in the permeability of the mesentery could cause pathological damage to the kidney, and the kidney damage might be reduced through the repair of the mesentery using the anti-TLR4 or/and GEC treatments.

For the analysis of glomerular IgA deposition, the team used a tissue immunofluorescence technique. The sedimentation of IgA in the glomerulus is the direct cause of IgAN. By measuring the sedimentation of IgA with immunofluorescence technology, the therapeutic effects of a drug used for kidney disease can be determined.

For detection of protein expression in mesenteric lymphoid tissues—toll-like receptor 4 (TLR4), B-cell activating factor (BAFF), and a proliferation-inducing ligand (APRIL), the team used western blot analysis. The expression of TLR4, BAFF, and APRIL can directly affect the amount and activity of B cells, which indirectly leads to the overexpression and sedimentation of IgA.

Outcome Measures

BUN. Post-intervention BUN was higher in the intervention group than in the IgAN group, elevated BUN implies impaired renal function

Signal molecules for serum inflammation. Inflammatory factor was higher in the intervention group than in the IgAN group after the intervention, and elevated inflammatory factor implies increased inflammatory damage.

IgA+B220+B lymphocytes. IgA+B220+B lymphocytes were higher in the intervention group than in the IgAN group after the intervention, and elevated IgA+B220+B lymphocytes imply an intense immune-inflammatory reaction.

Pathological damage in kidney tissue. Kidney tissue damage was higher in the intervention group than in the IgAN group after the intervention, and elevated kidney tissue damage implies impaired kidney function

Glomerular IgA deposition. Post-intervention IgA levels were higher in the intervention group than in the IgAN group, and elevated IgA implies increased renal impairment

Protein expression. Urine protein levels were higher in the intervention group than in the IgAN group after the intervention, and elevated urine protein implies increased renal impairment

Statistical Analysis

The study used SPSS 22.0 software (IBM, Armonk, NY, USA) for the statistical analysis. The research team expressed the study's results as means ± standard deviations (SDs). The team used the paired t test for within-group comparisons and applied the two independent sample *t* test to compare the relapse scores between the groups. $P < .05$ was considered to be statistically different. The research team produced the figures using Origin 2020 software (IBM, Electronic Arts Inc, Redwood City, CA, USA).

RESULTS

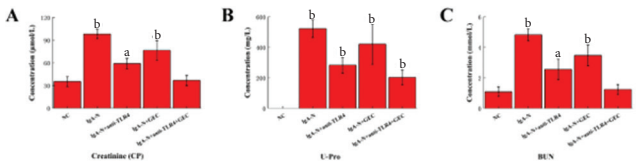
Specific Physiological Indicators

The kidney function of the mice in the NC group was normal, with no detection of urinary protein in the animals' urine, while the blood creatinine, urinary protein, and urea nitrogen of the IgAN group was significantly higher ($P < .001$). Blood creatinine, urine protein and urea nitrogen levels were higher in the IgAN group than in the three intervention groups. (Figure 1).

Inflammatory Factors

For the IgAN group, the inflammatory factors IL-6, TNF-α, MCP-1, COX-2, and Gd-IgA1 were all significantly

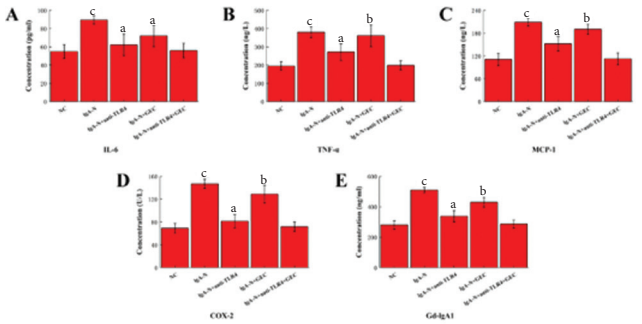
Figure 1. Creatinine, Urea Protein, and Blood Urea Nitrogen (BUN) Using ELISA. The data for the NC group are all equivalent to that of healthy mice. Figure 1A shows creatinine; Figure 1B shows urea protein; and Figure 1C shows blood urea nitrogen.



[a]$P < .01$, BUN levels were significantly higher in the intervention group than in the IgAN group after the intervention
[b]$P < .001$,Urea protein levels were significantly higher in the intervention group than in the IgAN group after the intervention.

Abbreviations: Anti-TLR4, TLR4 antibody; ELISA, enzyme-linked immunoassay; GEC, glutamine enteric-coated capsules; IgAN, IgA nephropathy; NC, normal controls; TLR4, toll-like receptor 4.

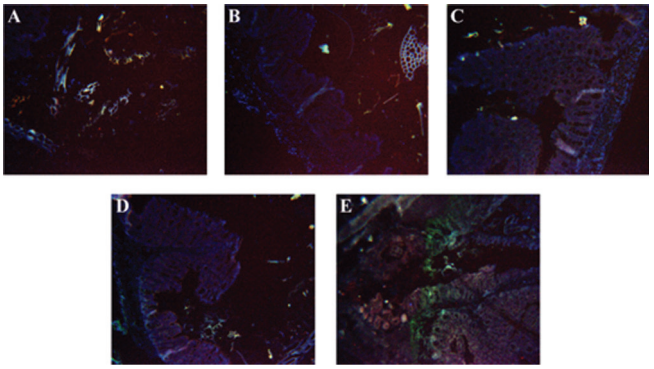
Figure 2. Inflammatory Factors IL-6, TNF-α, MCP-1, COX-2 and Gd-IgA1 Using ELISA. The data for the NC group are all equivalent to that of healthy mice. Figure 2A shows IL-6; Figure 2B shows TNF-α; Figure 2C shows MCP-1; Figure 2D shows COX-2; and Figure 2E shows Gd-IgA1.



[a]$P < .05$, indicating that postintervention the IgAN+anti-TLR4 group and the IgAN+GEC group had significantly higher levels of IL-6 than the IgAN group did and that the IgAN+anti-TLR4 group had significantly higher levels of TNF-α, MCP-1, COX-2 and Gd-IgA1 than the IgAN group did
[b]$P < .01$, indicating that postintervention the IgAN+GEC group had significantly higher levels of TNF-α, MCP-1, COX-2 and Gd-IgA1than the IgAN group did
[c]$P < .001$, The levels of each inflammatory factor were significantly higher in the intervention group than in the IgAN group after intervention

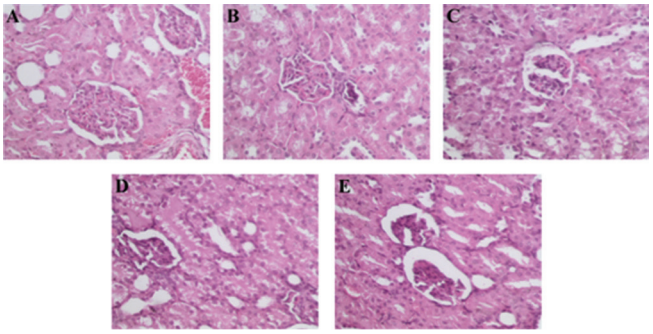
Abbreviations: Anti-TLR4, TLR4 antibody; COX-2, cyclooxygenase-2; Gd-IgA1, galactose-deficient IgA1; GEC, glutamine enteric-coated capsules; IgA, immunoglobulin A; IgAN, IgA nephropathy; IL-6, interleukin-6; MCP-1, monocyte chemotactic protein 1; NC, normal controls; TNF-α, tumor necrosis factor alpha; TLR4, toll-like receptor 4.

Figure 3. The Immunofluorescence Results of Intestinal Tissues. The luminous points in the pictures are the target IgA+B220+B lymphocytes. Figure 3A shows the NC group; Figure 3B shows the IgAN group; Figure 3C shows the IgAN+anti-TLR4 group; Figure 3D shows the IgAN+GEC group; and Figure 3E shows the IgAN+anti-TLR4+GEC group.



Abbreviations: Anti-TLR4, TLR4 antibody; B220, B-cell marker; GEC, glutamine enteric-coated capsules; IgA, immunoglobulin A; IgAN, IgA nephropathy; NC, normal controls; TLR4, toll-like receptor 4.

Figure 4. The HE Staining Results for Kidney Tissues. The white parts in the pictures are the pathological damage caused by IgAN. Figure 4A shows the NC group; Figure 4B shows the IgAN group; Figure 4C shows the IgAN+anti-TLR4 group; Figure 4D shows the IgAN+GEC group; and Figure 4E shows the IgAN+anti-TLR4+GEC group.



Abbreviations: Anti-TLR4, TLR4 antibody; GEC, glutamine enteric-coated capsules; HE, hematoxylin-eosin; IgA, immunoglobulin A; IgAN, IgA nephropathy; NC, normal cells; TLR4, toll-like receptor 4.

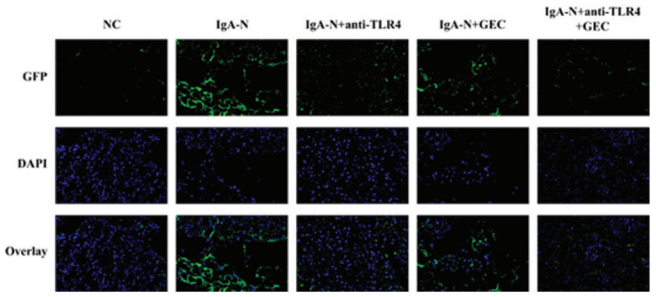
higher than that of the NC grip, and the level indicates a strong inflammatory response in the body. The research team found that the inflammation mainly occurred around the mesentery and glomerulus.

For the IgAN+anti-TLR4, IgAN+GEC, and IgAN+anti-TLR4+GEC groups, the inflammatory responses were also significantly higher than those of the NC group, although they were lower than that of the IgAN group. Among those three groups, the levels of inflammatory factors in the IgAN+anti-TLR4+GEC group postintervention were at a normal level, which indicates the best therapeutic effect (Figure 2).

IgA+B220+B Lymphocytes in Intestinal Tissue

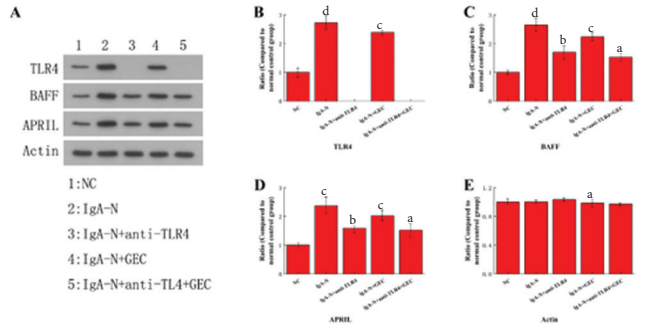
Figure 3 shows the content of IgA+B220+B lymphocytes in the intestinal tissue. With the IgAN group showing the highest content, the comparison of the content of IgA+B220+B lymphocytes in the groups shows: IgAN group> IgAN+GEC

Figure 5. The Immunofluorescence Results of Kidney Tissues. The blue fluorescence in the pictures are the IgA sediment in glomerulus. The trend of IgA sediment is as following: IgAN group> IgAN+GEC group> IgAN+anti-TLR4 group> IgAN+anti-TLR4+GEC group> NC group.



Abbreviations: Anti-TLR4, TLR4 antibody; DAPI, 4',6-diamidino-2-phenylindole; GEC, glutamine enteric-coated capsules; GFP, green fluorescent protein; IgA, immunoglobulin A; IgAN, IgA nephropathy; NC, normal cells; TLR4, toll-like receptor 4.

Figure 6. The Western Blot Results of the Proteins TLR4, BAFF, APRIL, and Actin in Mesenteric Lymphoid Tissues. Figure 6 A shows the original gel electrophoresis image, and Figures 6B-E show the ratio of protein expression compared to NC group.



[a]$P < .05$, indicating that postintervention the IgAN+GEC group had significantly higher levels of BAFF and April than the IgAN group did
[b]$P < .01$, indicating that postintervention the IgAN+anti-TLR4 group had significantly higher levels of BAFF and April than the IgAN group did
[c]$P < .001$, indicating that postintervention the IgAN group had significantly higher levels of April than the NC group did and that the IgAN+GEC group had significantly higher levels of TLR4, BAFF, and April than the IgAN group did
[d]$P < .0001$ indicating that postintervention the IgAN group had significantly higher levels of TLR4 and BAFF than the IgAN group did

Abbreviations: Anti-TLR4, TLR4 antibody; APRIL, a proliferation-inducing ligand; BAFF, B-cell activating factor; GEC, glutamine enteric-coated capsules; IgA, immunoglobulin A; IgAN, IgA nephropathy; NC, normal controls; TLR4, toll-like receptor 4.

group> IgAN+anti-TLR4 group> IgAN+anti-TLR4+GEC group> NC group.

Pathological Damage

Figure 4 shows the characteristics of the pathological damage to kidney tissue. The figure indicates that the degree of kidney damage from high to low was as follows: IgAN group> IgAN+GEC group> IgAN+anti-TLR4 group> IgAN+anti-TLR4+GEC group> NC group. The simultaneous use of anti-TLR4 and GEC made the kidney damage almost the same as NC group.

IgA Sedimentation

Regarding the benefits on three interventions with respect to sedimentation, Figure 5 shows that the use of GEC only resulted in the worst outcome; the use of anti-TLR4 only was second, and the use of both at the same time produced the best outcomes.

TLR4, BAFF, and APRIL

The Western blot analysis fully confirmed the inhibitory effects of anti-TLR4 and GEC on the three immune-related proteins (Figure 6).

DISCUSSION

Overall, the TLR4 antibody and GEC for the treatment of intestinal tract regulated and repaired intestinal function, so that IgA nephritis was also relieved at the same time. The results supported the hypothesis that a relationship exists between IgAN and the LPS/TLR4 pathway that regulates mesenteric B cells to secrete low-glycosylated poly-IgA1, which provides a new potential therapeutic plan for IgA nephritis.

AUTHOR CONTRIBUTIONS

Haidong He, Meilin Shen, and Yuyan Tang should be considered Co-first author.

ACKNOWLEDGMENTS

There are no conflicts of interest in this study.

AUTHORS' DISCLOSURE STATEMENT

The study received funding from the Science and Technology Commission of Shanghai Municipality of the project of Shanghai Key Laboratory of Kidney and Blood Purification (No.14DZ2260200), the National Natural Science Foundation of China (No.81774060), and the Scientific research project of Shanghai Health and Family Planning Commission(No.20184Y0040). The research team declares that they have no competing interests.

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