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Original article

Immune effects of miRNA and Th17 cells on β-Lg allergy in dietary milk based on mouse model

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ABSTRACT

Objective: β-lactoglobulin (β-Lg) allergy in dietary milk seriously affects the use of high-quality milk protein in infants. In order to solve this problem, the expression of miRNA and Th17 cells in milk β-Lg allergic reaction of children's diet was studied. Method: female BALB/c mice aged 5–6 weeks were selected as the subjects and randomly divided into blank group and β-Lg sensitized group, with 10 mice in each group. On the 1st, 7th and 14th day, the mice in the β-Lg sensitized group were intraperitoneally injected with allergen (Freund's adjuvant + β-Lg). Mice in the blank group were given the same amount of normal saline. Blood samples were collected from the eyeballs of mice to determine the number of inflammatory cells. The contents of Th17 related cytokines and transcription factors in spleen were detected by RT-PCR. Results: 1. the number of eosinophils and neutrophils in the β-Lg sensitized group were 15.76/mL and 24.36/mL, respectively, which were significantly higher than those in the blank group ($P < 0.05$); 2. in the mice of β-Lg sensitized group, the expression of miR-146a and miR-155 was abnormal, the number of Th17 cells was abnormally increased, and the expression levels of IL-17 and RORγt were significantly increased; 3. the abnormal expression of miR-146a and miR-155 in the mice of β-Lg sensitized group was positively correlated with the secretion of Th17 related cytokines, which could be used as one of the biological indexes to evaluate allergic reaction. Conclusion: the number of Thl7 increased abnormally in dietary milk allergy patients. miRNA gene expression and IL-17 expression could be used as one of the biological indicators to evaluate the allergic reaction of β-Lg.

1. Introduction

Milk is a key source of food protein for infants and young children because of its high nutrient content (Kianmehr et al., 2017; Pacholewska et al., 2017). According to epidemiological investigation, 2% ~ 6% of children are troubled by immunoglobulin E-mediated type I hypersensitivity induced by β-Lg in milk (Liu et al., 2017; Zhang et al., 2018). Hypersensitivity is the response of organism to persistent stimulation of antigen or repeated stimulation of the same antigen. It is mainly manifested in physiological disorder and tissue damage (Jlds et al., 2017; Tangye et al., 2017). The allergic reaction of milk is due to the linear and conformational allergen epitopes of the amino acid sequence of allergen proteins that can bind to specific antibodies. After a series of immune reactions, various allergic diseases such as gastrointestinal tract, respiratory tract or skin are formed (Cortesperez et al., 2017).

T lymphocyte subsets play a key role in allergic reaction. T-helper (Th) cells play a key role in immune regulation in the pathogenesis of food allergy. According to the different biological functions and cytokines secreted, Th mainly includes the identified Th1 and Th2 cells and the newly discovered regulatory T cells (Treg) and Th17 cells. Their interaction and coordination constitute a complex immune network to maintain the immune balance of the body.

In recent years, more and more studies have pointed out that it is necessary to explore the mechanism of Th17 dysfunction in β-Lg allergy (Perez et al., 2020). It is also pointed out that a class of non-protein-coded miRNA are involved in innate immunity, adaptive

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immunity regulation and signal transduction of inflammatory factors, and are an important regulator of Th17 cell proliferation, differentiation and function (Iwamoto et al., 2020; Torri et al., 2017).

Now, the research of miRNA in the pathogenesis of allergy is still in its infancy. Further study on the correlation between miRNA and Th17 cells is of great significance for supplementing and improving the immunological pathogenesis of food allergy and exploring targeted therapy in this link (Montoya et al., 2017; Wu et al., 2017). Therefore, an animal model of β-Lg sensitization was established to detect the number of Th17 cells and the expression of related cytokines in serum and spleen of β-Lg allergic mice, so as to clarify the changes of Th17 cells and miRNA gene expression in sensitized mice, improve the immunological pathogenesis of dietary milk allergy, and provide a new target for immunotherapy after dietary milk allergy.

2. Materials and methods

2.1. Experimental subjects and primer sequences

Experimental subjects: BALB/c female mice, with clean grade and 5–6 weeks old, are purchased from Beijing Weitong Lihua Laboratory Animal Technology Co., Ltd., China. Feeding temperature is maintained at 23 ± 2 °C and relative humidity is 55 ± 5%. Artificial light is used to illuminate the environment for 12 h every day. They are fed without β-Lg and free to drink water.

The primers needed in this experiment are synthesized by Shanghai Biotechnology and Bioengineering Co., Ltd., China (Table 1).

Table 1
Primer sequence.

Gene	Primer sequence	
miR-146a	Forward	5-CGGCGGTGAGAACTGAATTCCA-3
	Reverse	5-ATCTAGTGGAGTGTACGTGG-3
miR-155	Forward	5-AAGCGGCGGTTAATGCTAATTGTGAT-3
	Reverse	5-ATACGCTGCACGGACCTACG-3
IL-17	Forward	5-GCAGGCACAAACTCATCCAT-3
	Reverse	5-GGGCGAAAATGGTTACGAG-3
RORγt	Forward	5-TGATAGATAAGGCGAACTCA-3
	Reverse	5-GAGTAGTGGATGATGGGATC-3

2.2. Extraction of lymphocyte from mouse spleen and synthesis of cDNA

Female BALB/c mice of 5–6 weeks are fed adaptively for 2–3 days. The mice are killed by cervical dislocation and immersed in 75% alcohol for 2–3 min to reduce hair contamination. The mice are moved into the super clean worktable. The abdomen is cut and the spleen of the mice is removed and put into sterile PBS. The sterile spleen is put into 10 mL RPMI-1640 culture medium containing 10% fetal bovine serum. Splenocyte suspension is prepared by injecting spleen cells with syringe. Then, the cell suspension is collected and centrifuged for 5 min at 1000r, and the supernatant is removed. 2–3 mL erythrocyte lysate is added, placed for 4–5 min, and centrifuged for 5 min at 1000r/min. The supernatant is removed, and 10 mL RPMI-1640 culture medium is added, washed twice and suspended again. RPMI-1640 complete medium is used to regulate the cell density to 2–5*106/mL, and the cell count is carried out with a blood count plate.

Total RNA extraction: Mouse spleen lymphocytes are extracted and cultured with β-Lg in 37 °C and 50% CO2 incubator for 24 h. After centrifugation for 5 min, the liquid in the incubation pore is taken for 1500r/min. The precipitation is the sample, and then the total RNA is extracted strictly according to the total RNA extraction kit of Tiangen cells.

The specific steps of synthesizing cDNA are as follows. Template RNA is thawed on ice. 5 × gDNA Buffer, FQ-RT Primer Mix, 10 × Fast RT Buffer, and RNase-Free dd H2O are thawed at room temperature and quickly placed on ice after thawing. Before use, each solution is whirlpool-oscillated and centrifuged briefly to collect the residual liquid on the wall of the tube. Mixture is prepared according to the genomic DNA removal system in Table 2 and blended thoroughly. It is centrifuged briefly, incubated at 42 °C for 3 min and placed on ice. Mix in reverse transcription reaction should be added to the reaction solution of gDNA removal step to mix well. It is incubated for 15 min at 42 °C, and placed on ice at 95 °C for 3 min. The obtained DNA can be used for subsequent experiments or cryopreservation.

Table 2
Composition of RT reaction solution.

Reagent	Usage quantity	Final concentration
5 * gDNA Buffer	3 μL	1 *
TotaL RNA	7 μL	
RNase-Free ddH_2O	3 μL	

2.3. Establishment of mouse model

The healthy and clean BALB/c female mice aged 5–6 weeks with ± 10 g body weight, are randomly divided into blank group and β-1g sensitized group (10 mice in each group) after 3 days of adaptive feeding. As shown in Fig. 1, the allergens (1 mL Freund's adjuvant +1 mL 1 mg/mLβ-1g) are injected intraperitoneally on the 1st, 7th and 14th day in the β-1g sensitized group, and the blank group is given the same amount of saline at the same time. After sensitization on the 1st and 7th days, eyeball blood is collected and serum is collected for reserve. On the 16th day, 5 mg β-1g is used for the last oral stimulation, and blood, colon, lung and spleen are collected at different time intervals (0.5 h, 1 h, 1.5 h and 3 h) after stimulation.Fig. 2.

2.4. Extraction of total RNA from mouse spleen and colon tissues

Extraction of total RNA from mouse spleen and colon tissues: Mouse spleen and colon tissues are powdered with liquid nitrogen, and poured into centrifugal tube, and RZ lysate is added. The nucleic acid-protein complexes can be completely separated when the mixed samples are placed at room temperature for 5 min. After centrifuging for 5 min at 4 °C 12000r (~13400*g), the supernatant is extracted and put into a new centrifugal tube. 200 μL chloroform is added to the sample, covered, shaking violently for 15 s, and placed at room temperature for 3 min. After centrifugation for 10 min at 4 °C and 12000r (~13400*g) the sample will be divided into three layers, which are yellow organic phase, intermediate layer and colorless aqueous phase. RNA is mainly in the aqueous phase, the volume of which is about 50% of the RZ reagent used in the pyrolysis solution. The water phase is transferred to the new pipe for further operation. About 0.5 times the volume of the sample, anhydrous ethanol is slowly added. After shaking, the solution and precipitation are moved to the adsorption column together. After centrifugation at 4 °C and 12000 rpm (~13400 *g) for 30 s, if all the solutions and mixtures cannot be added to the column at one time, they can be transferred into the column twice. After centrifugation at 4 °C and 12000 rpm (~13400 *g) for 30 s, the waste liquid in the collecting pipe is dumped. The 500 μL deproteinized solution RD, which has been added to absolute ethanol, is added to the adsorption column. After centrifugation at 4 °C and 12000 rpm (~13400 *g) for 30 s, the waste liquid is dumped.

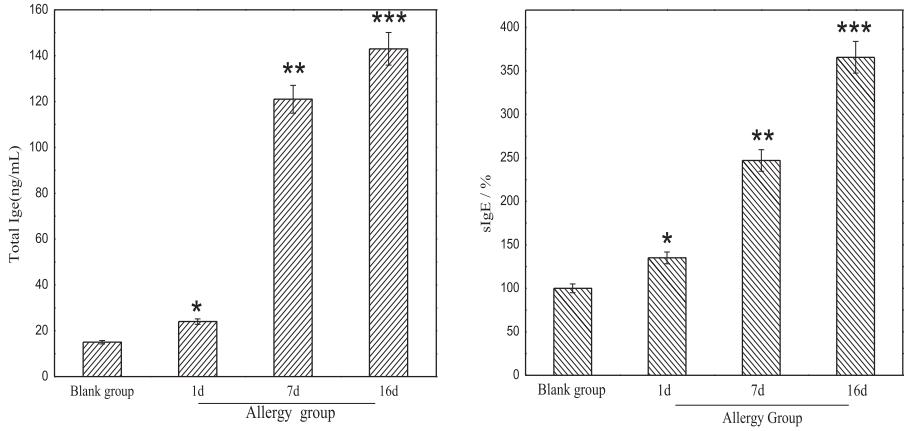

Fig. 1. Contents of total IgE and sIgE in serum of β-Lg sensitized model (* compared with the blank group, P < 0.05; ** compared with the blank group and the 1st day of the same group, P < 0.05; *** compared with the blank group and the 1st day and 7th day of the same group, P < 0.05).

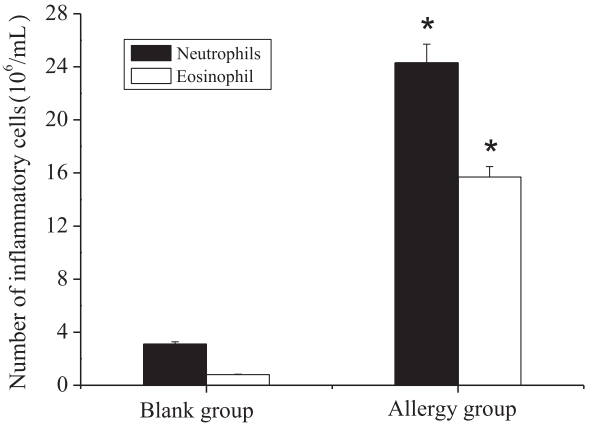

Fig. 2. Changes of inflammatory cells in blood of β-Lg sensitized model (* compared with the blank group, P < 0.05).

CR3 is put in collection tube. The rinse solution RW 500 μL, which has been added to absolute ethanol, is added to the adsorption column and placed for 2 min. After centrifugation at 4 °C and 12000 rpm (~13400 *g) for 30 s, the waste liquid is dumped. The adsorption column is placed in a 2 mL collecting tube. After centrifugation at 4 °C and 12000 rpm (~13400 *g) for 2 min, the remaining liquid is removed. The adsorption column is transferred into a new 1.5 mL centrifugal tube, and 40 μL RNase-Free ddH_2O is added. It is placed at room temperature and centrifuged for 2 min at 4 °C 12000r (~13400 *g).

2.5. Detection of Th17 cells in spleen by flow cytometry

After sterilized in 75% alcohol, mice are removed and placed on a dish with scissors and tweezers, with the left abdomen facing up. Alcohol cotton is used to wipe the abdomen of mice and a pairlarge dissecting scissors is used to cut the abdomen along the abdomen towards the neck. Another pair of scissors is used to take out the spleen, and remove the surrounding adipose tissue. PBS is used to wash it for 1–2 times, then it is transferred to a dish containing PBS. The 1 mL syringe is used to absorb PBS and inject it into the spleen. After PBS effluent, the cell fluid is collected and centrifuged for 7 min at 1500r. The erythrocyte lysate is added, and it is placed for 5 min, centrifuged and washed twice with PBS. The cell concentration is adjusted to 2 * 10^8 cells/mL.

100 μL prepared cell suspension is selected and the number of cells is about 2 * 10^7. 1 μL immunoglobulin C region (Fc) blocking solution is added and incubated at 4 °C for 15 min. 0.25 μL CD4 monoclonal antibody labeled with FITC and 0.3 μL CD25 antibody labeled with PE is added, and it is incubated at 4 °C for about 30 min. 1 mL pre-cooled PBS scrubbing cells are added. 1 mL fixed/breaking working fluid (1:3 dilution) is added after swirling heavily suspended cells. After vortex mixing again, incubation is conducted at 4 °C for 1 h. The cells are washed twice by centrifugation with 1 mL breaking buffer working fluid (1:10 dilution). Then, the supernatant is discarded and 100 μL breaking buffer working fluid is added. 1 μL Fc sealing fluid is added, and incubation is conducted at 4 °C for 15 min. 2.5 μL PE-CY5.5 labeled IL-17A antibody is added and incubated for 30 min at 4 °C. 1 mL membrane-breaking Buffer working fluid is added. Cells are centrifuged and washed, and supernatants are discarded. The cells are washed again and suspended by adding 0.5 mL PBS. Flow cytometry is used to detect and analyze the cells.

2.6. Detection of Th17-related cytokines and transcription factors in spleen by RT-PCR

According to the instructions of Tiangen total RNA extraction kit, the operation is strictly carried out. The specific process is as follows.

The spleen of mice frozen in liquid nitrogen is taken out about 50 mg. The spleen is ground into powder by liquid nitrogen grinding method and transferred to centrifugal tube containing 1 mL lysate RZ. After standing at room temperature for about 5 min and the nucleic acid protein complex is completely separated, the supernatant is placed in a new centrifugal tube. 200 μL chloroform is added, the centrifugal tube cap is sealed, and the centrifugal tube is shaken violently for about 15 s. It is placed at room temperature for 3 min. After centrifugation for 10 min at 4 °C, the samples can be divided into yellow organic phase (lower layer), middle layer and colorless aqueous phase (upper layer). The water phase is sucked into the new tube with a liquid pipette for the next operation, and then 0.5 times volume of anhydrous ethanol is slowly added. After mixing evenly, all the solutions (possibly containing precipitation) are transferred to the adsorption column CR3, and the adsorption column is put into the collection tube. After centrifugation for 30 s, the waste liquid in the collection tube is discarded. The 500 μL protein-free liquid RD (adding ethanol before use) is added to the adsorption column. After centrifugation for 30 s, the waste liquid in the collection tube is discarded. 600 μL ethanol-added rinse RW is added to the adsorption column, and it

is kept at room temperature for 2 min. After centrifugation for 30 s, the waste liquid in the collection tube is discarded. The adsorption column is placed in the collecting tube and centrifuged for 2 min to remove the residual liquid. The adsorption column is transferred into a new centrifugal tube and 50 μL Rnase-Free ddH_2O is added. The column is placed at room temperature for 2 min and centrifugation is carried out for 2 min.

RNA is detected by agarose gel electrophoresis: 1 g agarose is selected and measured at 100mLTAE (1 *). It is taken into the electrophoresis room with 6 * loading buffer, pipette and gun head. Agarose and TAE are poured into a triangular bottle and heated several times in a microwave oven until they melt. A drop of Eb dye is added, the solution is poured into the rubber tank, and the bubbles are driven away by gun until they solidified. After gelation, it is put into electrophoresis tank and poured into TAE to immerse the gel. On PE gloves, a small drop of loading buffer (about 3 μL) is taken, and an equal amount of RNA is added and blended. Sample on gel: On the first hole, the sample is pointed as Marker, and other holes are pointed samples. The power supply is switched on. After about 0.5 h, the power supply is turned off according to the strip condition. The glue is put into the imaging room to observe whether RNA is extracted or not. If RNA is extracted, RT-PCR reaction is performed.

RT-PCR reaction: The operation is carried out strictly according to the instructions of the bio-fluorescent PCR kit, and the reaction liquid is prepared according to Table 3 (It is better to prepare the reaction liquid on ice.). Then, the RT-PCR reaction is carried out with ABI System 7500.

2.7. Detection of various indexes in mice

The general signs are as follows.

The allergic symptoms of the whole body are observed within one hour after gastric administration, and the clinical scores are made according to the symptoms (0–5 points). 0: Asymptomatic. 1: There are traces of scratching nose and head in mice. 2: There are swelling in eyes and head, diarrhea and upright hair, decreased activity and increased respiratory frequency. 3: Mice suffer from respiratory distress, asthma, cyanosis of mouth and tail. 4: There is no reaction and convulsion after touching. 5: Death. At the same time, the weight changes of mice are observed.

Two mice in each group are randomly selected to collect blood from their eyeballs. Serum is collected after centrifugation. The total IgE concentration in the serum of mice is determined strictly according to the instructions of R&DELISA. The kit detection method is as follows.

The dilution and sample addition of standard product: 10 holes are marked on the enzyme-coated plate, 100L of standard product is added in holes 1 and 2 respectively, and then 50 μL of standard diluent is added in holes 1 and 2 to mix. From holes 1 and 2, 100 μL is added to holes 3 and 4 respectively, and then 50 μL standard diluent is added to holes 3 and 4 respectively to mix evenly. In holes 3 and 4, 50 μL is taken and discarded, 50L is added to holes 5 and 6 respectively, and 50 μL standard diluent is added to holes 5 and 6 respectively to mix evenly. After mixing evenly, 50L from holes 5 and 6 is added to holes 7 and 8 respectively, and 50 μL standard diluent is added to holes 7 and 8 respectively. After mixing evenly, 50L is taken from holes 7 and 8 and added to holes 9 and 10 respectively, and then 50 μL of standard diluent is added to holes 9 and 10 respectively. After mixing evenly, 50L is taken from holes 9 and 10 and discarded (After dilution, the dosage of each pore is 50 μL, the concentration is 900 ng/mL, 600 ng/mL, 300 ng/mL, 150 ng/mL, and 75 ng/mL, respectively.). Sampling addition: The blank hole (No samples and enzyme-labeled reagents are added to the blank control pore, and the other steps are the same) and the sample hole to be measured are set respectively. 40 μL sample diluent is added to the pore of the sample to be tested on the enzyme labeling plate, and then 10 μL sample to be tested is added (The final dilution of the sample is 5 times). Samples are added to the bottom of the pore of the enzyme label plate. The pore wall should not be touched and the sample is shaken gently. Temperature incubation: Enzyme labeling plate is sealed with sealed plate membrane and cultured in 37 °C incubator for 30 min. Distribution: 20 times concentrated detergent is diluted with distilled water 20 times and reserved. Washing: The sealing film is slowly opened, the liquid inside is poured out, and then dried. Each hole is filled with the detergent, and it is placed for 30 s. Then, it is poured out. After five times of repeated washing, it is patted dry with absorbent paper. Enzyme addition: 50 μL enzyme labeled reagent is added to every pore except blank pore. Incubation and washing are carried out. Colour development: 50 μL colour developer A is added to each pore, then 50 μL colour developer B is added. It is slowly shaken and mixed. At 37μ, 15 min of color rendering without light is carried out. Termination: 50 μL termination solution is added to each pore to terminate the reaction (blue turns yellow vertically at this time). Determination: The absorbance (OD) of each hole is measured sequentially at zero and 450 nm wavelength of blank air conditioner. The determination should be carried out within 15 min after adding termination solution.

The detection of sIgE is as follows.

Coating specific antigen: 100 μL antigen solution (10ug/mL β-1g solution) is added to each concave hole, and the liquid in the hole is discarded after overnight at 4 °C and washed three times. Closure: 200 μL sealing liquid is added to each hole. The lid is added and it is incubated in 37 °C incubator for 60 min. The plate is washed three times. The 100 μL sample is added to the corresponding concave hole. The lid is added and it is incubated in 37 °C incubator for 60 min. The plate is washed three times. Enzyme-labeled antibodies: 100 μL (1:50000) HPR-sheep anti-rat solution is added, and 100 μL is added to each hole, and then sealed in 37 °C incubator for 60 min. The plate is washed three times. Colour development: 100 μL TMB colour solution is added to each hole, and reaction plate is placed in room temperature darkness for 15 min at 37 °C. Termination reaction: 50 μL sulfuric acid solution is added to each pore, the reaction pore changes from yellow to brown, and the colorimetric determination can be carried out after stabilization for 3–5 min.

The eyeballs of mice are removed for blood collection, and the number of inflammatory cells is determined by the following steps.

A drop of blood is dripped on one end of the clean slide. A slide with smooth edge is used as a slider. The slider is pulled back slightly at an angle of 30–45°from the blood drop, and moved left and right to form a line of blood droplets, and adhere to the edge of the slide. The pushing tablet is pushed smoothly from one end to the other end with uniform force until the blood is exhausted. After the blood tablets are pushed, they are kept at room temperature to dry completely. Dyeing: Crayons are used to draw lines at both ends of the blood sheet to prevent the dye from flowing out. Blood

Table 3
Composition of reaction solution.

Reagent	Usage quantity	Usage quantity	Final concentration
PCR Forward Primer (10uM)	11 μL	25 μL	1 *
RT reaction solution (DNA solution)	2.5 μL*2	5 μL	
dH_20 (Sterilized distilled water)	6 μL	18 μL	
Total	22 μL*4	48 μL*4	

Reaction conditions of PCR.
Predenaturation: 95 °C, 30 s, 1 cycle; Denaturation: 90 °C, 5 s, 40 cycles; Annealing/elongation: 60 °C, 30 s, 40 cycles.

tablets are placed on the platform, and 3 to 5 drops of Swiss dye are dripped to cover the whole blood membrane. After 1 min, the same amount of phosphate buffer is added. At room temperature, the staining is carried out for 10 min and it is rinsed with water. The stained blood sheets are upright in the air, waiting for microscopic examination.

Inflammatory cell count: it is observed and counted under the microscope, in which eosinophils are bright red, and neutrophils are light red purple or pink.

2.8. HE dyeing and data processing

Fixation: After the cervical dislocation of mice is executed, the colon of mice is taken after immersion and disinfection in 75% alcohol. The contents are removed. The colon of mice is washed with saline, and weighed with filter paper. It is fixed in 10% formalin for 24 h. Preparation of wax blocks: Gradient alcohol is used to dehydrate (70% – 85% – 95% – 100%) for about 6 h, xylene is transparent for 20 min, and wax is dipped three times with 1 h each time. Tissue is embedded and sliced (5um), and the slices are baked. Dewaxing: Xylene is continuously used for 3 times, 5 min each time. Rehydration: Gradient alcohol is used for 2 min (100% – 95% – 80% – 70%) and distilled water is used for cleaning. Dyeing: Hematoxylin-eosin is used for dyeing. Hematoxylin is used for dyeing for 5 min, the differentiation solution is used for 10 s. After washing with tap water, it is placed in the blueback solution for 10 s, tap water is used for washing, eosin is used for 10 s, and tap water is used for washing.

Dehydration: Gradient alcohol is used for 2 min (100% – 95% – 80% – 70%). Transparent: Xylene solution is used for washing twice, 10 min each time. Seal: Neutral gum is used to seal the colon and then dried to observe the pathological morphology of mouse colon under optical microscope.

The obtained data are processed by SPSS19.0. One-way ANOVA is used for comparison between groups, LSD is used for comparison between two groups, and Microsoft Excel 2007 software is used for correlation analysis. $P < 0.05$ shows significant difference.

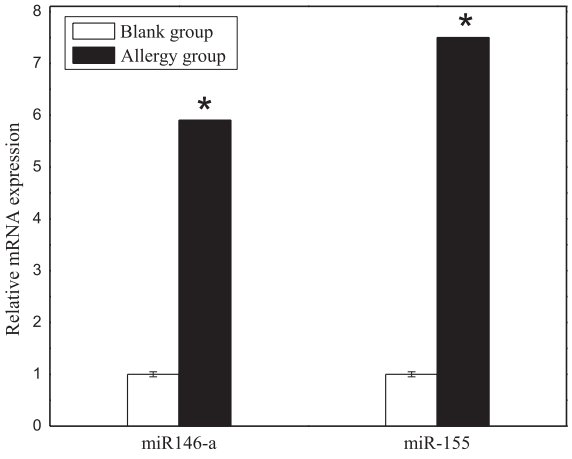

Fig. 3. Differential expression of miRNA in splenic lymphocytes of β-Lg allergic mice (* compared with the blank group, $P < 0.05$).

3. Results and discussion

3.1. Verification results of β-Lg allergic animal model

As shown in Fig. 1, the total IgE and sIgE titers of mice in the β-Lg sensitized group increase significantly on the 7th day after β-Lg stimulation. On the 16th day, the total content of 1gE in the serum of sensitized mice reaches 145.1 ng/mL, which is significantly higher than that of the blank group (12.5) ($P < 0.05$), and the titer of sIgE increases to 365.7% (the percentage of OD value in the blank group).

From Table 2, it can be seen that the number of eosinophils and neutrophils in the plasma of allergic group is 15.76/mL and 24.36/mL, respectively, which is significantly higher than that of blank group (0.86/mL and 3.16/mL) ($P < 0.05$). After sensitization with β-Lg, compared with normal saline control group, both the lung and colon of sensitized mice have inflammatory cell infiltration. Alveolar wall of lung tissue becomes thicker and blood vessel dilates, which indicates that the β-Lg allergic model of BALB/c mice is successfully established.

3.2. Expression of miRNA in lymphocytes and tissues of β-Lg allergic mice

After killing mice, splenic lymphocytes are extracted and co-cultured with 1 mg/mL of β-Lg for a period of time. RT-PCR is used to analyze the expression of different miRNA in lymphocytes stimulated by β-Lg. The results are shown in Fig. 3. Compared with the blank group, the expression of miR-146a and miR-155 in splenic lymphocytes stimulated by β-Lg increases significantly ($P < 0.05$), and the up-regulation of miR-155 is relatively high, which is 7 times higher than that in the blank group ($P < 0.05$), followed by miR-146a, which is 6 times higher than that in the blank group ($P < 0.05$), with statistical significance.

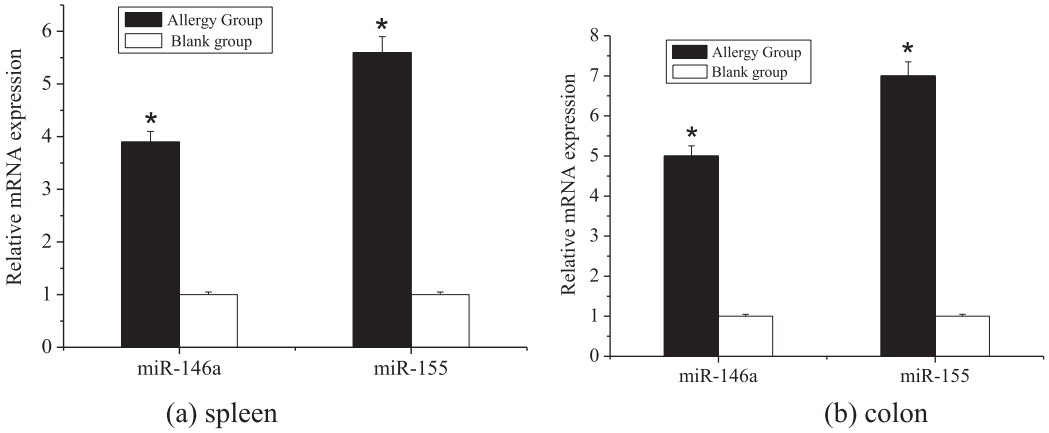

Fig. 4. Differential expression of miRNA in spleen and colon of β-Lg allergic mice (* compared with the blank group, $P < 0.05$).

RT-PCR is used to detect the difference of expression of miR-146a and miR-155 in spleen and colon of normal mice and allergic mice. Results is shown in Fig. 4. Compared with the blank group, the levels of miR-146a and miR-155 in spleen and colon tissues are significantly increased ($P < 0.05$), and the magnification of miR-155 is the highest, which is 5 times higher in spleen tissues than that in blank group and 7 times higher in colon tissues than that in blank group.

3.3. Changes of Th17 cells in spleen of β-1g allergic mice

To determine the changes of Th17 cells, $CD4^+$ and Th17 cells in spleen of blank group and allergic mice are detected. As shown in Fig. 5, the Th17 content of mice sensitized by β-Lg is 4.58% higher than that of the blank control group (0.89%) ($P < 0.05$). It is concluded that β-Lg sensitization can lead to imbalance of Th17 cells.

3.4. Changes of Th17-related cytokines and transcription factors in β-1g sensitized model

Fig. 6 shows that, in the serum of mice sensitized by β-1g, the concentration of IL-17A (28.4 ng/L), which is the key cytokine of Thl7, is significantly higher than that of the blank group (15.1 ng/L) ($P < 0.05$). From Fig. 7, it can be seen that intraperitoneal injection of β-1g can significantly increase the expression of Th17 cell-related transcription factor ROR gamma t in mice ($P < 0.05$).

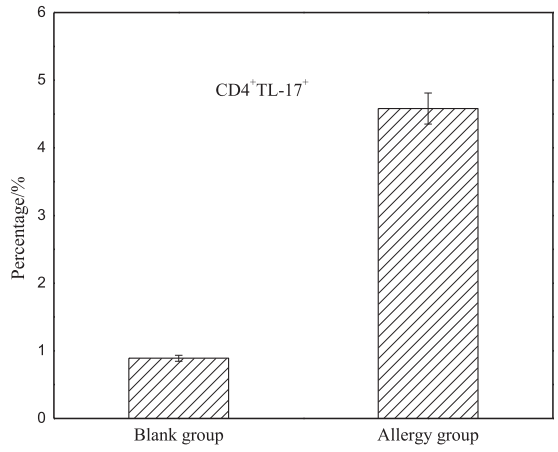

Fig. 5. Percentage of Thl7 in CD4 cells.

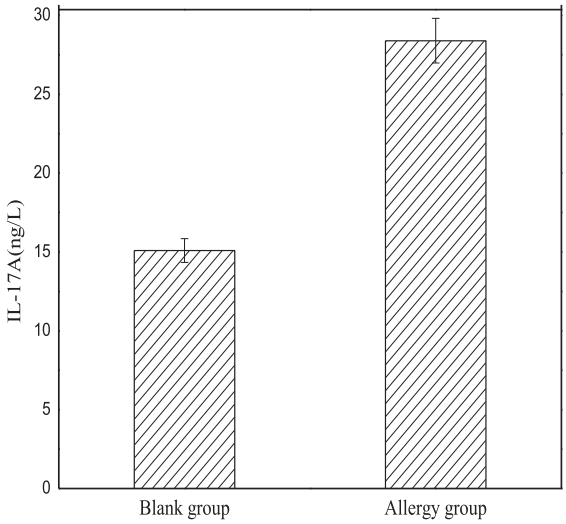

Fig. 6. Changes of Th17 cytokines in β-1g sensitized model.

3.5. Analysis of the correlation between miRNA and Th17 cells in β-Lg allergic mice

The correlation between Th17-related cytokines and transcription factors and the expression of miRNA is further analyzed. As shown in Fig. 8, there is a significant positive correlation between the expression levels of miR-146a mRNA and miR-155 mRNA in spleen tissue of allergic mice and IL-17mRNA and RORγt mRNA.

4. Conclusion

Recent studies have indicated that miRNA is involved in innate immunity, adaptive immunity regulation and signal transduction of inflammatory factors. At the same time, it is necessary to explore the mechanism of Th17 dysfunction in β-Lg allergy. In this study, BALB/c female mice for 5–6 weeks are used as the subjects. The expression of miRNA in spleen lymphocytes of β-Lg allergic mice is detected by extracting spleen lymphocytes from mice. At the same time, the mouse model is established and the contents of Th17-related cytokines and transcription factors in spleen are detected by RT-PCR. BABL/c mice model was established by intraperitoneal injection of β-Lg. It is found that the IgE level of sensitized mice is significantly higher than that of the blank control group, and the number of eosinophils in the blood of mice in sensitized group is also significantly higher than that of the blank group. The changes of these two indexes are consistent with the research results of Sun et al. (Sun et al., 2018).

To sum up, the immune effects of miRNA and Th17 cells in children's dietary milk allergy to β-Lg are studied based on mouse model, and the results are as expected. The number of Thl7 increases abnormally in dietary milk allergy patients. MiRNA gene expression and IL-17 expression can be used as one of the biological indicators to evaluate the allergic reaction of β-Lg. This study provides reliable experimental data for investigating the immune effects of miRNA and Th17 cells in milk allergic reaction in children. However, there are still some shortcomings in this study, such as less data acquisition in the experimental process, which makes its mechanism cannot be accurately explored. Therefore,

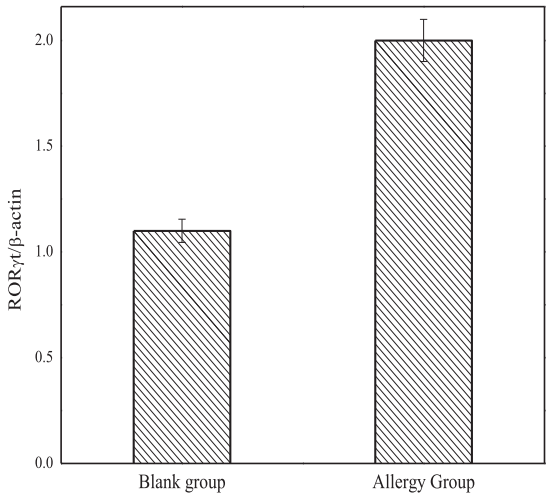

Fig. 7. Changes of transcription factors in Th17 cells in a β-1g sensitized model.

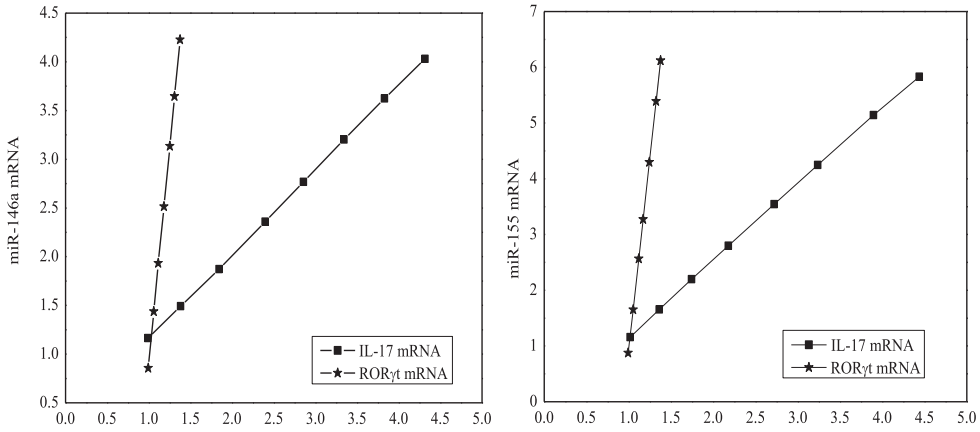

Fig. 8. Analysis of the correlation between miRNA and Th17 cells.

in the future work, it is necessary to increase data capacity to further explore its mechanism.

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