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Curative effect of BCG-polysaccharide nuceic acid on atopic dermatitis in mice

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ABSTRACT

Objective: To explore the effect of bacilli Galmette-Gurin (BCG)-polysaccharide nuceic acid on atopic dermatitis in mice and its mechanism. **Methods:** Forty NC/Nga mice were selected and randomly divided into Group A (model group), Group B (dexamethasone treatment group), Group C (BCG polysaccharide nucleic acid treatment group) and Group D (control group) with 10 mice in each group. Atopic dermatitis model were constructed by applying 2, 4-dinitrochlorobenzene on the skin of the mice. Mice in Group D were treated with acetone solution (100 μL) on the foot pad and abdomen after hair removal at the age of 7 weeks, then on ear skin at the age of 8-13 weeks. For mice in A, B and C groups, 100 μL of acetone solution containing 2, 4-dinitrochlorobenzene was applied to the foot pad and the abdomen at the age of 7 weeks, then on ear skins at the age of 8 to 13 weeks. At the age of 7-13 weeks, mice in Group A and Group D were treated with 100 μL saline (*i.p.*); mice were given dexamethasone (0.1 mL/kg, *i.p.*) every other day for 7 weeks in Group B; mice were treated with BCG polysaccharide nucleic acid (0.5 mg/kg, *i.p.*) every other day for 7 weeks in Group C. The ear thickness was measured every week and the scratching frequency was recorded 1 times for 10 min a week. The mice were sacrificed after the last administration of drugs. IgE, IL-4, IL-10, IL-12 and IFN-γ in the plasma were detected using ELISA, and RT-PCR method was employed to detect the concentrations of IL-4, IL-10, IL-12 and IFN-γ proteins. After HE staining, the lesion degree of inflammation in ear tissue was observed microscopically. **Results:** The ear thickness and scratching frequency of Group A were significantly higher than those in group B, C and D ($P<0.05$), and there was no significant difference between Group B and C ($P>0.05$); the concentrations of IgE, IL-4 and IL-10 in the plasma and the expression of IL-4, IL-10 mRNA in the spleen tissues of Group A, B and C were all significantly higher than those of Group D ($P<0.05$); the concentrations of plasma IL-12 and IFN-γ, and spleen protein expression of IL-12 and IFN-γ in Group C mice were significantly higher than those of Group A ($P<0.05$). Histological observation showed obvious ear tissue exudation, erythema, swelling, desquamation of skin, and scabbing in Group A. Histopathology of the skin lesion also showed hyperkeratosis, focal-parakeratosis, stratum spinosum hypertrophy, mild sponge-like edema, a large number of lymphocytes along with plasma cell infiltration in dermis, angiectasis and hyperemia in Group A, while degree of ear skin lesion in Group B and D mice was significantly lighter than that of Group A. **Conclusions:** BCG polysaccharide nucleic acid can significantly reduce the serum IgE concentrations, increase the expression of IL-12, IFN-γ protein, correct the imbalance of Thl/Th2 in atopic dermatitis mice, and has obvious inhibitory effect on atopic dermatitis in NC/Nga mice.

1. Introduction

Atopic dermatitis is a common clinical skin disease which is often associated with allergic disease[1-3]. According to statistics[4-6], about 60% of atopic dermatitis occurs in the first year of infant period after birth. Skin lesion in childhood was often detected in crimple, back and the extensor aspects of the limbs, with manifestations of ichenification, invasive plaque. During the adolescence and adulthood, lichen-like skin lesion can often be

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found at flexor sides of the limbs, head and neck. Skin lesions can cause different degrees of itching and seriously affect the patient's normal sleeping and quality of life[7]. At present, the pathogenesis of atopic dermatitis is not fully understood and the recurrence rate is high, which makes the treatment more difficult. Therefore, it is of great importance to study the pathogenesis and therapy of atopic dermatitis. Some studies have confirmed that[8], Thl/Th2 cells act their immunization effects through the secretion of different cytokines. Normally, cytokines produced by Th1 and Th2 act through the mutual antagonism of the function of each other, achieving balance; but in the skin lesion and blood of patients suffered from atopic dermatitis type Th2 cytokines prevail over Th1 cytokines. Based on the above equilibrium theory, the authors consider that adjusting the balance of Th1/Th2 cytokines and inhibiting the dominant response of Th2 will show a positive effect on the treatment of atopic dermatitis. BCG polysaccharide nucleic acid is a novel immunomodulator extracted from BCG. It is effective in treatment of asthma, allergic rhinitis, chronic measles and other allergic diseases. It also has therapeutic effect on atopic dermatitis[9]. In the present study, to explore the treatment effect of BCG polysaccharide nucleic acid on atopic dermatitis in mice and its mechanism, NC/Nga mice was selected for constrction of atopic dermatitis model and intraperitoneal injection of BCG polysaccharide nucleic acid treatment was given.

2. Materials and methods

2.1. Experimental animals

Forty specific pathogen-free (SPF) NC/Nga mice, regardless of gender, at the age of 6 weeks, weighing 10-23 g were purchased from Charles River Laboratories, Japan. The mice were made to acclimatize to the environment by clean and full-nutrient feeding at temperature (23±3) ℃ and humidity (50±10)%. Animals were allowed to have free access to diets and water with lighting from 8 am to 8 pm every day. The dealing of experimental animals was performed according to Regulations for the Administration of Affairs Concerning Ex-Perimental Animals.

2.2. Apparatuses and reagents

BioTek Elx 800 automatic plate reader and fluorescent inverted microscope were produced by Gene companies (USA); Rotor-Gene 3000 fluorescence-based quantitative PCR was produced by Corbett Company (Australia). 2, 4-Dinitrochlorobenzene, IL-4, IL-10, IL-12 and IFN-γ primers were provided by Shanhai Yingjun Biotechnology Co., LTD. (China). BCG polysaccharide nucleic acid was procured from Jiuzhi Tang, Siqi Biological Pharmaceutical Co., LTD (Hunan, China). Acetone, dexamethasone and sodium sulfide were from Chemical Reagent Factory of Hunan Normal University (Hunan, China). Mice, IgE, IL-4, IL-10, IL-12, IFN-γ and ELISA kits were purchased from Invitrogen (USA).

2.3. Construction of atopic dermatitis and experiment protocols

Forty NC/Nga mice were selected and randomly divided into Group A (model group), Group B (dexamethasone treatment group), Group C (BCG polysaccharide nucleic acid treatment group) and Group D (control group) with 10 mice in each group. Atopic dermatitis model in mice were constructed by applying 2, 4-dinitrochlorobenzene on the skin of the mice. At the age of 7 weeks, mice in Group D were treated with acetone solution (100 μL) on the foot pads and abdomens after hair removal; and at the age of 8-13 weeks, acetone solution (100 μL) was applied on ear skin of the Group D mice. For mice in Group A, B and C, at the age of 7 weeks, 100 μL of acetone solution containing 5% 2, 4-dinitrochlorobenzene was applied to the foot pad and the abdomen after removal of hair; and at the age of 8 to 13 weeks, 100 μL acetone solution containing 2, 4-dinitrochlorobenzene (0.1%) was applied once a week on their ear skins. At the age of 7-13 weeks, Group A and Group D mice were treated with 100 μL saline (*i.p.*); Group B mice were given dexamethasone (0.1 mL/kg, *i.p.*) every other day for 7 weeks; Group C mice were treated with BCG polysaccharide nucleic acid (0.5 mg/kg, *i.p.*) every other day for 7 weeks.

2.4. Observation

The ear thickness of the mice in each group was measured every week and the scratching frequency was record 1 times for 10 min every week. The mice were sacrificed after the last administration of drugs. Blood was withdrawn and concentrations of IgE, IL-4, IL-10, IL-12 and IFN-γ in the plasma were detected using ELISA. After resection of the spleens, RT-PCR method was employed to detect the concentrations of IL-4, IL-10, IL-12 and IFN-γ proteins in each group; ear tissues were resected for preparation of tissue slice. After HE staining, the lesion degree of inflammation in ear tissue was observed microscopically.

2.5. Statistical analysis

Results were processed by using SPSS17.0 statistical software, measurement data were expressed as mean±SD. Statistical difference was determined by One-way ANOVA and student's t test, $P<0.05$ was considered as statistically significant.

3. Results

3.1. Ear thickness comparison

The ear thickness of Group A mice at the age of 8-13 weeks was significantly higher than that of Group D mice (P<0.05). At the age of 9-13 weeks, ear thickness of mice in both Group B and C was significantly higher than that of Group D, but lower than that of Group A (P<0.05); and ear thickness in Group C were lower than that of Group B (P>0.05) (Table 1).

3.2. Scratching frequency of mice in each group at different time point

The scratching frequency of Group A, B and C mice was significantly higher than that of Group D mice (P<0.05); while scratching frequency of Group B and C was significantly lower than that of Group A (P<0.05); and the scratching frequency of Group C was significantly lower than that of Group B (P<0.05) (Table 2).

3.3. Concentrations of plasma IgE, IL-4, IL-10, IL-12 and IFN-γ in each group

Concentration of IgE in plasma was significantly higher than that of the other three groups (P<0.05); IgE concentration was significantly higher in Group B and C than that of group D (P<0.05); concentration of IL-4 and IL-10 in plasma in Group A was significantly higher than that in Group D (P<0.05), while the levels of IL-12 and IFN-γ in Group A mice showed no significant statistical difference compared with those of Group D (P>0.05); the concentration of IL-12 and IFN-γ in plasma of Group C mice were significantly higher than that of Group D (P<0.05), but IL - 4, IL-10 in the plasma of Group C mice showed no statistical difference compared with those of Group A (P>0.05); IL-4, IL-12 and IFN-γ in the plasma of Group B mice was significantly lower than that of group D, and L-10 concentration was significantly higher (P<0.05) (Table 3).

3.4. Expression of IL-4, IL-10, IL-12, IFN-γ mRNA in the spleens of mice in each group

IL-4, IL-10 mRNA expression in the spleen tissues of Group A mice was significantly higher (P<0.05) and IL - 12 mRNA expression was lower (P<0.05) than that of group D, and IFN-γ mRNA expression showed no statistical difference compared with that of Group D (P>0.05). After treatment, IL-12 and IF-γ mRNA expression in spleen tissue of Group C mice was significantly higher than that of Group A (P<0.05), but IL-4 and IL-10 expression showed no statistical difference compared with group A (P>0.05). IL-4 and IFN-γ mRNA expression in Group B mice was significantly lower than that of Group A (P<0.05), while IL-10 was significantly higher than that of Group A (P<0.05), and IL-12 showed no statistical differences compared with that of Group A (P>0.05) (Table 4).

Table 1

Ear thickness of mice in different group (mm).

Group	n	7 weeks	8 weeks	9 weeks	10 weeks	11 weeks	12 weeks	13 weeks
A	10	0.30±0.03	0.35±0.02*	0.43±0.03*	0.52±0.03*	0.61±0.06*	0.64±0.06*	0.78±0.04*
B	10	0.29±0.02	0.32±0.02	0.35±0.03#	0.40±0.04#	0.46±0.03#	0.44±0.03#	0.52±0.17#
C	10	0.30±0.03	0.31±0.02	0.35±0.03#	0.39±0.02#	0.42±0.03#	0.43±002#	0.49±0.03#
D	10	0.30±0.02	0.30±0.08	0.31±0.01	0.31±0.01	031±0.02	0.32±0.01	0.32±0.02

*P<0.05 compared with Group D, #P<0.05 compared with Group A.

Table 2

Scratching frequency of mice in each group at different time point.

Group	n	7 weeks	8 weeks	9 weeks	10 weeks	11 weeks	12 weeks	13 weeks
A	10	2.6±0.6*	5.0±1.2*	7.8±1.3*	9.8±2.6*	12.2±2.8*	14.4±1.3*	14.8±1.3*
B	10	2.8±0.8*#	4.8±1.5*#	5.0±1.0*#	6.4±1.5*#	7.2±0.8*#	7.0±1.6*#	7.6±0.9*#
C	10	2.4±0.6*#△	3.6±1.5*#△	3.8±0.8*#△	5.8±1.3*#△	5.6±1.1*#△	6.6±1.3*#△	7.2±0.5*#△
D	10	1.0±1.0	0.8±0.8	0.4±0.1	0.6±0.9	1.0±0.7	1.0±0.7	1.0±0.7

*P<0.05 compared with Group D, #P<0.05 compared with Group A, △P<0.05 compared with Group B.

Table 3

Concentrations of plasma IgE, IL-4, IL-10, IL-12 and IFN-γ in each group (pg/mL).

Group	n	IgE (ng/mL)	IL-4	IL-10	IL-12	IFN-γ
A	10	174.7±12.64*	91.49±6.32*	50.97±1.67*	40.14±6.15	51.53±3.45
B	10	50.43±5.36*#	75.79±15.34*#	69.53±3.57*#	30.35±2.85*#	40.83±5.78*#
C	10	84.27±9.35*#△	88.44±12.69*	51.64±3.52*	122.10±4.64*#	73.89±2.39*#
D	10	17.32±3.56	83.95±6.63	43.92±l.37	42.79±3.99	53.26±4.33

*P<0.05 compared with Group D, #P<0.05 compared with Group A, △P<0.05 compared with Group B.

Table 4
Expression of IL-4, IL-10, IL-12, IFN- γ mRNA in the spleens of mice in each group.

Group	n	IL-4	IL-10	IL-12	IFN- γ
A	10	1.670±0.850*	0.942±0.458*	0.615±0.132*	51.53±3.45
B	10	1.243±0.451#	1.359±0.463#	0.649±0.319	40.83±5.78#
C	10	1.792±0.650	0.960±0.556	2.206±1.045#	73.89±2.39#
D	10	0.935±0.372	0.624±0.230	0.993±0.296	53.26±4.33

*P<0.05 compared with Group D, #P<0.05 compared with Group A, △P<0.05 compared with Group B.

3.5. Histological observation

Histological observation showed normal dermis in the skin tissues of Group D mice. Obvious focal-parakeratosis, hyperkeratosis, mild sponge-like edema, stratum spinosum hypertrophy, angiectasis and hyperemia, cell infiltration of a large number of lymphocytes and eosinophilic granulocyte around blood vessels were observed in Group A mice. While, in Group B and C mice only mild hyperkeratosis, milder inflammatory cell infiltration and thinner prickie cells (compared with Group A mice) were observed (Figure 1).

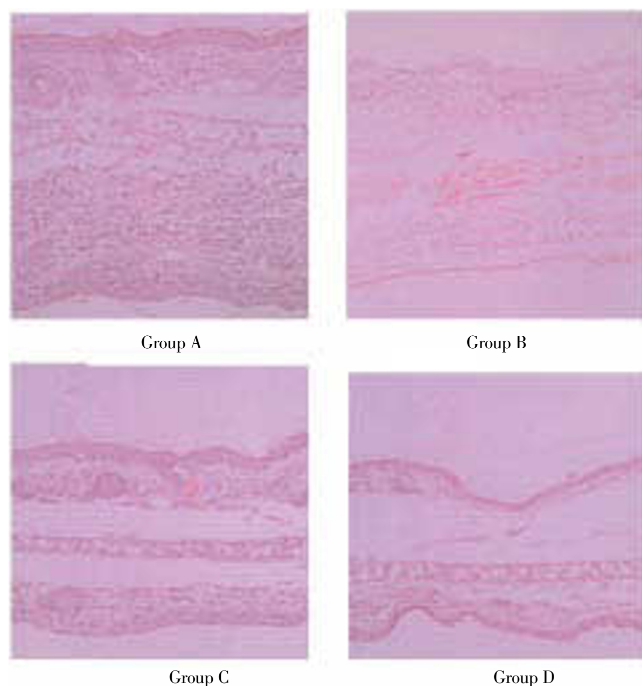

Figure 1. Histological observation of the ear skin of each group (HE, ×200).

4. Discussion

Atopic dermatitis, also known as atopic eczema, is a common clinical skin disease. Atopic dermatitis can cause different degree of pruritus at every stage of the disease and the pruritus can be more aggravating at night, seriously influencing the quality of the patient's sleeping and life. At present, the pathogenesis of atopic dermatitis is not entirely understood and the treatment for the disease is difficult with a high recurrence rate[10-13].

Some studies have confirmed that[14-16], Thl/Th2 cells play an immune role through the secretion of different cytokines. Normally, the cytokines produced by Th1/Th2 mutually antagonize with each other, achieving balance. But in the skin lesion and blood plasma of patients suffered from atopic dermatitis patients Th2 cytokines are in dominant position. Hence, the authors conclude that the imbalance of Thl/Th2 play a critical role in the pathogenesis of atopic dermatitis. Based on this theory, the authors consider that treatment for atopic dermatitis may be achieved by adjusting the imbalance between Thl/Th2 cytokines and inhibiting the response of Th2.

The main ingredients of BCG polysaccharide nucleic acid are lipopolysaccharide and nucleic acid. It is a bacteria lipopolysaccharide extracted from BCG vaccine after removing the protein. This will retain the immune function of BCG vaccine, and at the same time, reduce the adverse reaction. BCG polysaccharide nucleic acid is often used in clinical immunotherapy[17-19]. Studies have shown that[20-23], after intervention of BCG polysaccharide nucleic acid the activity of NF-KB and the expression of IgE decreased significantly in patients with atopic dermatitis, while the concentration of IFN- γ and IL-12 increases significantly, indicating that BCG polysaccharide nucleic acid can promote the expression of Thl and correct the imbalance state of Thl/Th2, thus, achieving the cure of atopic dermatitis. In the present study, after the treatment of BCG polysaccharide nucleic acid on atopic dermatitis mice in Group C, histological observation showed only mild hyperkeratosis, mild sponge-like edema, infiltration of a small amount of inflammatory cells, less skin lesion thickness than that of Group A mice and significantly decreased and scratching frequency (P<0.05), suggesting that BCG polysaccharide nucleic acid can effectively improve the skin lesion in mice with atopic dermatitis. In addition, at the end of BCG polysaccharide nucleic acid treatment the concentrations of IL-12 and IFN- γ in the plasma of Group C mice were remarkably increased, the concentration of IgE was decreased and IL-4 and IL-10 was not influenced, confirming that BCG polysaccharide nucleic acid can effectively promote the secretion of IL-12 and IFN- γ , inhibit the production of IgE, thus adjusting the imbalance

of Th1/Th2, realizing its treatment effect.

The results of the present study shows that BCG polysaccharide nucleic acid can remarkably improve the skin lesion, decrease the concentration of IgE in the plasma, increase the expression of IL-12 and IFN-γ protein and adjust the imbalance of Th1/Th2 in NC/Nga mice with atopic dermatitis. BCG polysaccharide nucleic acid has outstanding treatment effect on atopic dermatitis mice.

Conflict of interest statement

We declare that we have no conflict of interest.

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