Int J Clin Exp Med 2016;9(2):2555-2560
www.ijcem.com /ISSN:1940-5901/IJCEM0015581

Original Article

Effect of BCG-PSN adjuvant therapy on Th1/Th2 immune response in bronchial asthma children

Hailei Wang[1], Na Ma[2]

[1]*Department of PICU, Huaihe Hospital of Henan University, Kaifeng 475000, China;* [2]*Department of Neurology, Kaifeng Children's Hospital, Kaifeng 475000, China*

Received September 4, 2015; Accepted December 19, 2015; Epub February 15, 2016; Published February 29, 2016

Abstract: Objective: To investigate the effect of BCG-polysaccharide nucleic acid (BCG-PSN) adjuvant therapy on Th1/Th2 immune response in bronchial asthma children. Methods: 109 cases of bronchial asthma children receiving treatment in our hospital were selected in this study. They were randomly divided into the control group and observation group. The children in the control group were treated with oxygen uptake, relieving asthma, anti-inflammatory, anti-infection and other conventional therapies. The observation group was treated with BCG-PSN adjuvant intramuscular injection on the basis of the control group, 1 ml, twice a week, 12 weeks were taken as one course of treatment. After treatment, the efficacies of the two groups were determined, and the expressions of INF-γ and IL-4 were detected in the peripheral blood of the two groups by enzyme-linked immunosorbent assay; Th1/Th2 in peripheral blood mononuclear cells was analyzed using flow cytometry. Results: The clinical efficacies were 72.73% and 94.44% in the control and observation group respectively. The clinical efficacies showed statistical significance between the two groups ($P<0.05$). Before treatment, there was no statistical significance in the levels of INF-γ and IL-4 as well as Thl/Th2 between two groups ($P>0.05$); after treatment, the INF-γ level was increased, the IL-4 level was decreased and Th1/Th2 was increased. Among them, the variation amplitude was more significant ($P<0.05$). Conclusion: BCG-PSN can significantly improve the levels of INF-γ and IL-4 as well as Th1/Th2 immunologic balance in serum of bronchial asthma children, can effectively improve the clinical efficacy in bronchial asthma children.

Keywords: BCG-polysaccharide nucleic acid (BCG-PSN), bronchial asthma, Th1/Th2

Introduction

Bronchial asthma is the most common chronic disease in childhood. The repeated attack of bronchial hyper responsiveness and chronic inflammation is one of the characteristics of bronchial asthma children. There are immune imbalance and abnormal expression of inflammatory mediators in bronchial asthma patients. Thl/Th2 cellular immunity is the immune cell system which is closely related to allergic reaction and immune response, and involved in the regulation of many inflammatory mediators [1, 2]. In recent years, although the drugs to effectively prevent recurrent asthma appear, and the standardized treatment of asthma is popularized, the incidence and prevalence of asthma are not only declined, but also shows an increasing trend in global range year by year. The incidence of asthma and its severity are increasing in developing countries. Data showed that the cumulative incidence of asthma in China was 0.25%-4.63% [3]. The immune pathogenesis of asthma showed that the number of Th2 cells was increased rapidly in acute attack of asthma and showed hyperfunction. The immune response level of Thl cells was significantly decreased, which made Thl/Th2 balance deviate to Th2 direction. Therefore, correction of Thl/Th2 balance was the key to treat bronchial asthma [4]. BCG-polysaccharide nucleic acid (BCG-PSN) was dead BCG preparation extracted from Bacilli Calmette-Gurin by hot phenol method. With respect to the BCG, BCG-PSN removed protein components. The main component was lipopolysaccharide, accounting for about 70%~80%. The rest component was nucleic acid, which removed the infectivity and retained immunogenicity. Studies showed that BCG-PSN had significant

Table 1. Comparison of curative effects in two groups

Groups	Cases	Markedly effective	Effective	No effect	The total efficiency (%)
Observation group	54	32	19	3	94.44*
Control group	55	18	22	15	72.73

Note: *P<0.05.

effects on allergic asthma, allergic rhinitis, nephrotic syndrome, tumor and other immune diseases [5, 6]. In this study, 54 cases of bronchial asthma children received BCG-PSN injection treatment. The effect of BCG-PSN on Thl/Th2 immune balance was observed and its clinical efficacy was evaluated.

Subjects and methods

General data

109 cases of bronchial asthma children receiving treatment in our hospital were randomly selected from January 2013 to January 2015. All patients were in accordance with the standard of asthma diagnostic diagnosis of Chinese Medical Association. The children were randomly divided into the control group and observation group. There were 55 cases in the control group, including 31 cases of male and 24 cases of female; the age was 2-11 years old and the average age was (7.54±1.36) years; the course was 1.1-7.3 years and the average course was (4.21±0.81) years. There were 54 cases in the observation group, including 31 cases of male and 23 cases of female. The age was 2-12 years old and the average age (7.68±1.45) years; the course was 1.2-7.5 years and the average course was (4.28±0.85) years. Exclusion criteria: (1) Severe visceral disease combined with heart, lung and kidney function insufficiency; (2) With chronic cough and consumptive diseases; (3) With other infectious diseases; (4) Using glucocorticoid and other immunomodulators. There was no significant difference in state of an illness, gender, age, disease, height etc. between the two groups (P<0.05). This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Huaihe Hospital of Henan University. Written informed consent was obtained from all participants.

Treatment method

The children in two groups were treated with budesonide (AstraZeneca Pharmaceutical Co. Ltd., London, UK) aerosol inhalation, 200 μg, twice a day, combined with relieving asthma, anti-inflammatory and anti-infection treatments. The children in the observation group were treated with BCG-PSN (Antai Pharmaceutical Co. Ltd., Xi'an, China) adjuvant intramuscular injection on the basis of the control group, 1 ml, twice a week. 12 weeks were taken as a course of treatment.

ELISA

2 ml fasting venous blood was acquired from the children in two groups respectively in the morning before and after treatments, and centrifuged by 4000 rpm for 10 min. The levels of INF-γ and IL-4 were detected strictly according to ELISA kit (R&D Systems Inc, Minneapolis, MN, USA). 3 complex holes were set for each sample and standard substance. The OD value was measured at 492 nm.

Flow cytometry analysis

3 ml fasting venous blood was acquired from the children in two groups respectively in the morning before and after treatments. The peripheral blood mononuclear cells were collected by density gradient centrifugation method and subpackaged into 3 tubes after washed with PBS, namely homotype control group, detection tube of the control group and detection tube of the observation group. 20 μl CD4-PerCP mAb (BD Biosciences, New Jersey, USA) was added and incubated for 30 min away from light at room temperature. The sample was washed, fixed with 4% paraformaldehyde, placed for 20 min at room temperature and quietly placed for 10 min. Intraprep™ Permeabilization reagent was added. The sample was centrifuged. The supernatant was discarded and the cells were resuspended; 20 μL IL-4-PE mAb (BD Biosciences, New Jersey, USA) and 20 μL IFN-γ-FITC mAb (BD Biosciences, New Jersey, USA) were added in the detection tube. 20 μL homotype control antibody IgG1-FITC mAb (BD Biosciences, New Jersey, USA) and 20 μL IgG1-PE mAb (BD Biosciences, New Jersey, USA) were added in the homotype control tube. The samples were incubated for 1 h away from light, washed, resuspended and detected by flow cytometry.

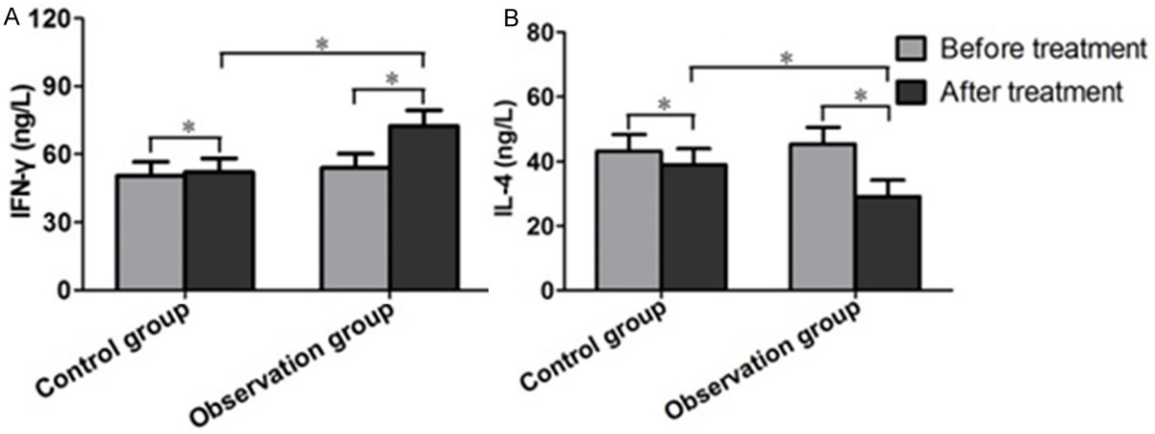

Figure 1. Comparison of INF-γ and IL-4 expression levels in peripheral blood between two groups. A. INF-γ expression levels in peripheral blood in two groups; B. IL-4 expression levels in peripheral blood in two groups.

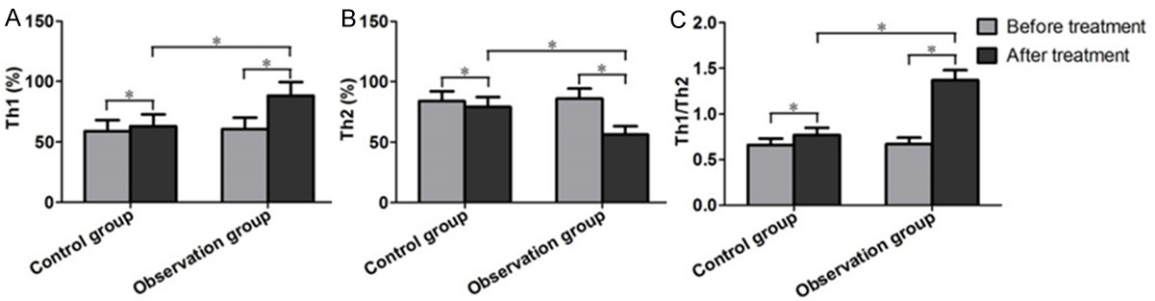

Figure 2. Comparison of Th1/Th2 in peripheral blood mononuclear cells between two groups. A. Th1 cell in peripheral blood mononuclear cells in two groups; B. Th2 ratio in peripheral blood mononuclear cells in two groups; C. Th1/Th2 ratio in peripheral blood mononuclear cells in two groups.

Efficacy determination

Efficacy determination criterion: Excellent: asthma symptoms were completely relieved during the treatment period and at the end of treatment; Effective: asthma symptoms were significantly relieved during the treatment period and at the end of treatment, the attack frequency was decreased, the course was shortened; Invalid: asthma symptoms were not relieved or was aggravated in the treatment period and at the end of the treatment. Total effective rate = (markedly effective rate + effective rate)/total number * 100%.

Statistical analysis

All data were analyzed by SPSS13.0 software (SPSS Inc, Chicago, IL, USA). The measurement data were represented with x ± sd. The measurement data were analyzed using t test. The enumeration data were represented with chi square test. $P<0.05$ indicated that the difference was statistically significant.

Results

Comparison of clinical efficacy between two groups

After treatment, the total effective rate was 94.44% in the observation group and 72.73% in the control group, the clinical efficacy of the observation group was significantly higher than that of the control group ($P<0.05$) (**Table 1**).

Comparison of INF-γ and IL-4 levels in peripheral blood between two groups

As shown in **Figure 1**, before treatment, the levels of IFN-γ and IL-4 in peripheral blood showed no significant difference between the control and observation group ($P>0.05$); After treatment, the IFN-γ levels were increased in the control group and observation group, the IL-4 level was decreased ($P<0.05$). Among them, the improved amplitude was more significant in the observation group ($P<0.05$).

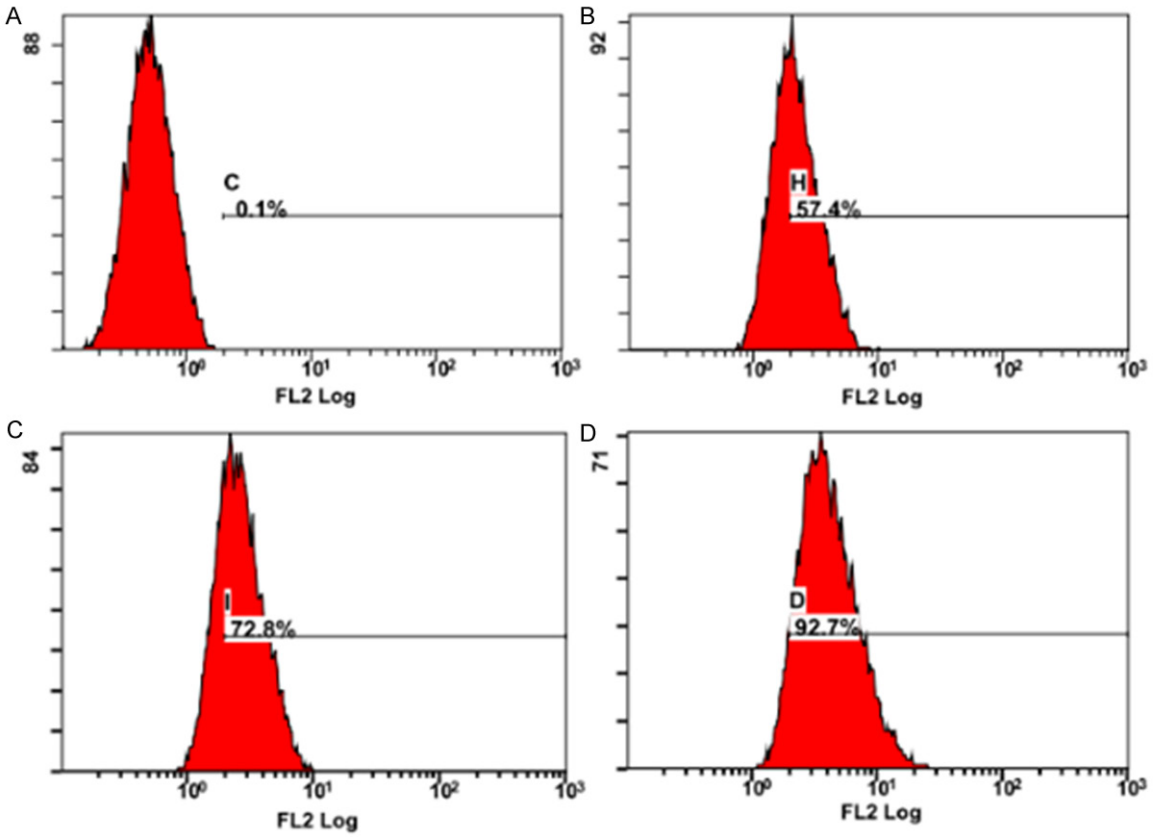

Figure 3. INF-γ expression levels in TH1 cells between two groups. A. Negative control; B. INF-γ expression levels in TH1 cells of children in two groups before treatment; C. INF-γ expression levels in TH1 cells in control group after treatment; D. INF-γ expression levels in TH1 cells in observation group after treatment.

Comparison of Th1/Th2 in peripheral blood mononuclear cells between two groups

As shown in **Figures 2**-**4**, Th1/Th2 in peripheral blood mononuclear cells showed no significance between the control and observation group ($P>0.05$); after treatment, Th1/Th2 of in peripheral blood mononuclear cells was increased in the control and observation group ($P<0.05$), and the rising range in the observation group was more significant ($P<0.05$).

Discussion

Bronchial asthma is an inflammatory airway disease. Acute inflammation can cause bronchial smooth muscle to contract and plasma to exude, which can lead to the acute onset of wheezing. Bronchial asthma is a chronic airway inflammation, in which many inflammatory cells such as eosinophils, mastocytes and T lymphocytes are involved. T lymphocyte activation and cytokines release have received more and more attention. T lymphocytes play an important immunomodulatory effect in the process of the disease, mainly Th1/Th2 subpopulation functional imbalance. A large number of researches proved that Th1/Th2 imbalance was the important immune pathogenesis.

Thl and Th2 subpopulations were differentiated from Th0 cells. Th1, Th2 and their secretase could mutually regulate proliferation and differentiation in normal physiological conditions, and thus to maintain the Th1/Th2 balance [9]. IFN-γ and IL-4 were the representative cytokines of Th1 and Th2 respectively. IFN-γ secreted by Th1, which could inhibit Th0 cells differentiation into Th2 cells, inhibit or reduce the accumulations of eosinophils and T lymphocytes in lung tissues; IL-4 secreted by Th2, which could induce B cells proliferation and differentiation into plasmocytes, induce the productions of IgG and IgE, further promote T cells differentiation into Th2 cells [10, 11]. When bronchial asthma occurred, the number of Th2

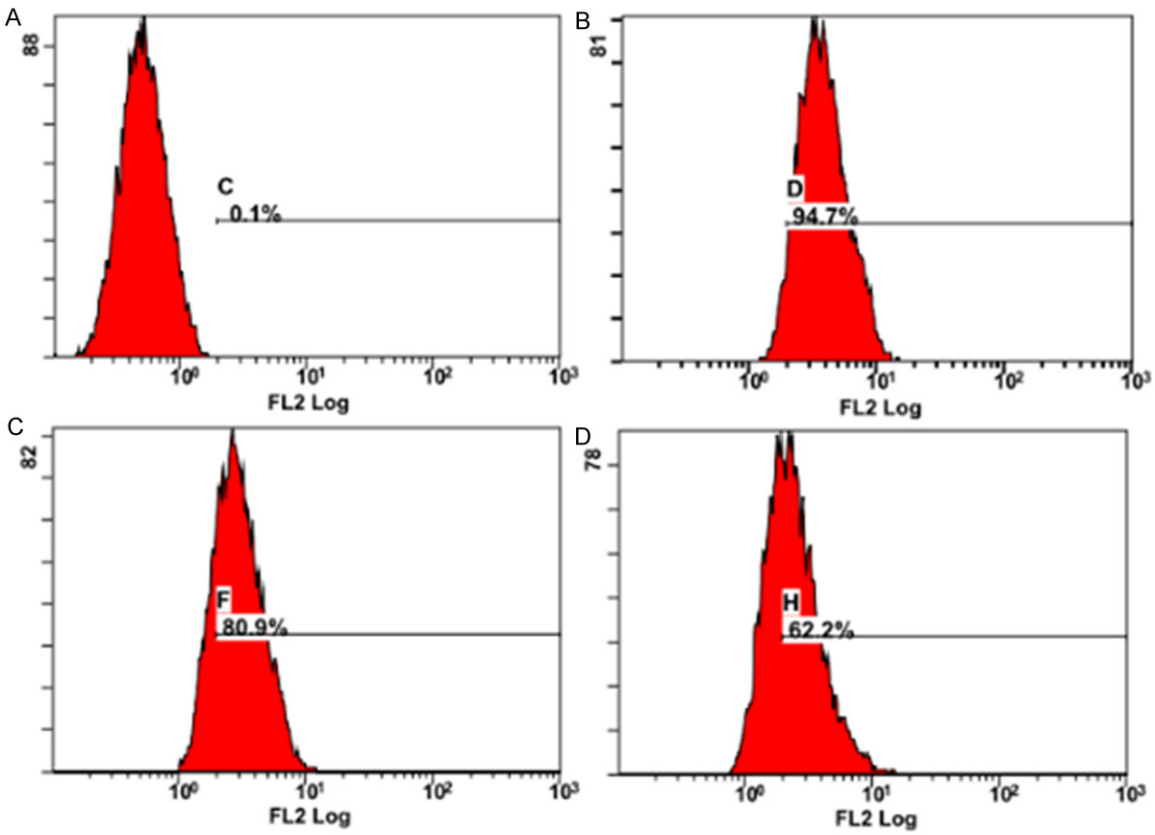

Figure 4. IL-4 expression levels in TH2 cells between two groups. A. Negative control; B. INF-γ expression levels in TH2 cells in two groups before treatment; C. INF-γexpression levels in TH2 cells in control group after treatment; D. INF-γ expression levels in TH2 cells in observation group after treatment.

cells increased, their function was activated, and combined with large secretions of IL-4 etc. Th2 type cytokines, which was a key factor to initiate and maintain airway inflammation [12]. Therefore, researchers generally agreed with Th2 cellular immunity hyperfunction in bronchial asthma children. Tang et al. [13] detected the levels of serum IL-4, IL-10 and IFN-γ in peripheral blood of 30 bronchial asthma patients in stage of attack and in remission stage. And the normal children were taken as the control group. The results showed that the levels of serum IL-4 and IFN-10 were higher than those in control group and remission group. The IFN-γ level was lower than that in control group and remission group. Th1/Th2 was lower than that in control group and IL-10 group. The results revealed that Th1/Th2 immune imbalance was one of pathogenetic mechanisms of bronchial asthma. The detection results of peripheral blood in 109 bronchial asthma children showed that the serum IFN-γ level was lower than normal reference value, the IL-4 level was higher than normal reference value, and Th1/Th2 was significantly lower than normal reference value. The results were consistent with previous reports, further proving the key role of Th1/Th2 immune imbalance in the pathogenesis of bronchial asthma.

BCG-PSN is an immunoregulatory substance composed of lipopolysaccharide and nucleic acid extracted from BCG. Bacillus Calmette Guerin (BCG) can be used for tuberculosis prevention. It was a strong Th1b immune revulsant and could induce Th1 cell proliferation and enhanced its function [14]. Studies showed that BCG-PSN had immunogenicity. It could inhibit the release of excessively hyperactive Th2 type cytokines after entered the human body. It could convert Th1/Th2 balance to Th1 type, which played a role in the prevention and treatment of asthma [15, 16]. In this study, 54 cases of bronchial asthma children were treated with BCG-PSN injection. The results showed that after treatment, the IFN-γ level in periph-

eral blood of patients in the observation group was significantly increased, the IL-4 level was decreased significantly ($P<0.05$) and Th1/Th2 of mononuclear cells in peripheral blood was increased significantly ($P<0.05$). The improved amplitude of all indexes showed significant difference compared with the control group ($P<0.05$). The research results showed that BCG-PSN could effectively reverse Th1 and Th2 cells immune imbalance, reverse the immune response from TH1 to Th2, which had important significance for bronchial asthma patients and was an effective way to treat bronchial asthma.

Acknowledgements

We are grateful to all the participants during performing this study.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Na Ma, Department of Neurology, Kaifeng Children's Hospital, No. 87 Ziyou Road, Kaifeng 475000, Henan Province, China. E-mail: mamahn@126.com

References

[1] Douros K, Loukou I, Boutopoulou B, Fouzas S. Does Vitamin D Deficiency Epidemic Parallel with Allergy and Asthma Epidemic? Mini Rev Med Chem 2015; 15: 967-973.

[2] Lebedenko AA, Semernik OE. Features of Autonomic Response in Children with Bronchial Asthma in the Period of Exacerbation. Vestn Ross Akad Med Nauk 2015; 2: 222-226.

[3] Dubey AK, Rajagopala S, Patel KS. Comparative clinical efficacy of Ashtangavaleha and Vyaghreehareetakee Avaleha on Tamaka Shwasa (bronchial asthma) in children. Ayu 2014; 35: 384-390.

[4] Al-Daghri NM, Alokail MS, Draz HM, Abd-Alrahman SH, Yakout SM, Clerici M. Th1/Th2 cytokine pattern in Arab children with severe asthma. Int J Clin Exp Med 2014; 7: 2286-2291.

[5] Ji JZ, Chen ZG, Chen YF, Chen FH, Chen H. Effects of BCG-PSN injection on immune and lung function of asthmatic children complicated with allergic rhinitis. Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi 2007; 21: 279-281.

[6] Song NN, Wang BX, Shi DZ, Fu LF, Luo Y, Yu HJ, Han SB, Jiao L, Qie YQ, Wang HH, Zhang Y, Zhu BD. Dimo-thylidioctyl ammonium bromide-BCG polysaccharide nucleic acid adjuvant enhanced the immunogenicity of a Mycobacterium tuberculosis subunit vaccine. Zhonghua Jie He He Hu Xi Za Zhi 2009; 32: 513-516.

[7] Huber M, Lohoff M. Change of paradigm: CD8+ T cells as important helper for CD4+ T cells during asthma and autoimmune encephalomyelitis. Allergo J Int 2015; 24: 8-15.

[8] Cai XJ. Effect of Yupingfeng particles on immunologic balance of Thl/Th2 in children with bronchial asthma. Chinese Journal of Practical Medicine 2014; 41: 9-11.

[9] Tang F, Wang F, An L, Wang X. Upregulation of Tim-3 on CD4 (+) T cells is associated with Th1/Th2 imbalance in patients with allergic asthma. Int J Clin Exp Med 2015; 8: 3809-3816.

[10] Chen SM, Tsai YS, Lee SW, Liu YH, Liao SK, Chang WW, Tsai PJ. Astragalus membranaceus modulates Th1/2 immune balance and activates PPARγ in a murine asthma model. Biochem Cell Biol 2014; 92: 397-405.

[11] Kau AL, Korenblat PE. Anti-interleukin 4 and 13 for asthma treatment in the era of endotypes. Curr Opin Allergy Clin Immunol 2014; 14: 570-575.

[12] Li J, Lin LH, Wang J, Peng X, Dai HR, Xiao H, Li F, Wang YP, Yang ZJ, Li L. Interleukin-4 and interleukin-13 pathway genetics affect disease susceptibility, serum immunoglobulin E levels, and gene expression in asthma. Ann Allergy Asthma Immunol 2014; 113: 173-179.

[13] Tang D, Liang P, Su PY. Changes of Thl/Th2 cell immune balance in children with bronchial asthma. Chinese Med Sci J 2011; 1: 43-44.

[14] Kawahara M, Matsuo K, Honda M. Intradermal and oral immunization with recombinant Mycobactefium bovis BCG expressing the simian immunodeficiency virus Gag protein induces long-lasting, antigen-specific immune responses in guinea pigs. Clin Immunol 2006; 119: 67-78.

[15] Liu SG, Feng YG, Wang HY. Efficacy of BCG-PSN in the prevention of recurrence of nephritic syndrome in children. Zhongguo Dang Dai Er Ke Za Zhi 2010; 12: 68-69.

[16] Sun J, Hou J, Li D, Liu Y, Hu N, Hao Y, Fu J, Hu Y, Shao Y. Enhancement of HIV-1 DNA vaccine immunogenicity by BCG-PSN, a novel adjuvant. Vaccine 2013; 31: 472-479.