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Original Research Article

Effect of immunotherapy on T lymphocyte subsets, FOXP3 and IFN-γ/IL-4 ratio in children with allergic asthma

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Abstract

Purpose: *To study the effect of specific immunotherapy on T lymphocyte subsets, FOXP3 and IFN-γ/IL-4 in children with allergic asthma (AA).*
Methods *57 AA children were selected and received the allergen specific immunotherapy, thereafter, the T lymphocyte subsets, FOXP3 and IFN-γ/IL-4 ratio were measured at three time points, before treatment, after 1yr and 2yr of treatment. Then the clinical efficacy after 2yr was recorded.*
Results *The pre- and post- treatment percentages of $CD4^+$ $CD25^+$ T cells in $CD4^+$ T cells had no difference (P>0.05). The proportion of $CD4^+$ $CD25^{high}$ T cells in $CD4^+$ T was significantly increased after 1yr and 2yr of treatment (P<0.05), the difference between that after 1yr and 2yr of treatment was not significant. The relative expression of FOXP3 at three time points in a descending order was after 1yr, 2yr of treatment and pre-treatment (P<0.05). The relative IFN-γ expression after 1yr of treatment increased and returned to pre-treatment level after 2yr of treatment; IL-4 expression levels decreased as the treatment time extended; The IFN-γ/IL-4 ratio at three time points in a descending order was after 1yr, 2yr of treatment and pretreatment (P<0.05). The levels of IL-4 and IFN-γ in the children increased significantly as treatment time extended (P<0.05), and became stable after 1yr and 2yr of treatment, while the concentration of IL-4 showed a decrease trend (P<0.05), also tended to be stable after 1yr and 2yr of treatment. The clinical efficacy after 2yr of treatment was 87.72%.*
Conclusion *This study has revealed that FOXP3, IFN-γ, and IL-4 have significant roles FOXP3 in the process of specific immunotherapy for children with allergic asthma. Further studies are needed to explore the mechanism.*

Key words *Specific immunotherapy; Allergic asthma; T lymphocyte subsets; FOXP3; IFN-γ/IL-4*

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INTRODUCTION

Allergic asthma (AA) is a chronic disease in the airways. Most occurrences are related to T lymphocytes, mast cells, eosinophils, various inflammatory cells and a series of cytokines [1]. Recently, the incidence rate of asthma in children has been enhanced by 3%, and is higher than that in adult (1%). The pathogenesis of the disease is still unclear, and the imbalance mechanism of TH1/TH2 ratio is mostly accepted, in which the excessive differentiation of TH2 leads to the imbalance of proportion [2]. $CD4^+$ $CD25^+$ T cells function in the pathogenesis of asthma [3]. The traditional treatments for asthma are mainly antispasmodic and anti-inflammatory, but the side effects of drug treatment are relatively severe. In addition, it is likely to recur after drug withdrawal, and the therapeutic effect is short-lived. Specific immunotherapy is also known as hyposensitization therapy or desensitization therapy [4]. Once the asthma allergen is clinically determined, and then the allergen extract is made into solution with different concentrations, and then the patients are repeatedly exposed to an allergen from low concentration to high, thereby improving the patient's tolerance, enabling the patient to produce immunity and no longer relapse [5]. The only therapy that may completely cure allergic asthma is allergen-specific immunotherapy. As medical technology advances, the effectiveness and safety of treatment methods have improved greatly, and thus received more and more attention [6].

This study was to explore the specific immunotherapy for asthma in children with dust mite allergies. By observing the changes of T lymphocyte subsets, FOXP3 and IFN-γ/IL-4 ratios in children, their role in the treatment process was discussed, in a bid to provide new methods and ideas for further treatments and research.

METHODS

Following ethical approval from the Ethic Committee of Binzhou Medical University, Binzhou, PR China, children (53), including 27 boys and 30 girls with mean age of 9.47 years, diagnosed with allergic asthama in our hospital from September 2014 to October 2015 were recruited for this study. These included children diagnosed with a history and symptoms of asthma, and met the diagnostic criteria for bronchial asthma. They equally met the specific immunotherapy indications and family members signed the informed consent agreement. Excluded were children with autoimmune or cardiovascular disease, severe asthma and those with history of specific immunotherapy.

First, the skin test for each child was carried out, and specific allergen the patient was reacting to was determined and preparations of allergens in different concentrations were prepared. The patients were exposed to the allergens by repeated subcutaneous injections, and the dose was increased continuously until the patients were again exposed to the allergens and no allergic reaction occurred or alleviated. immunotherapy was conducted using the dust mite allergen preparation. The specific immunotherapy (allergen, 20 units) was injection once a week and then increased weekly until the maximum dose of 100,000 units was reached at week 15. After reaching the maximum dose, the doses were injected once every other week. As the treatment time progressed, the changes in T cell subset, FOXP3, IL-4 and IFN-γ before treatment and at 1 year and 2 years after treatment were determined.

For the determination of the parameters, 3 ml of peripheral venous blood of the allergic children was drawn before and 1 year and 2 years after specific immunotherapy, and placed in EDTA anticoagulation tubes. The blood was separated by lymphocyte stratification density gradient centrifugation. Flow cytometry analysis was employed to measure the distribution of T cell subsets in patients. Fluorescence quantitative PCR was used to analyze the values of FOXP3, IL-4 and IFN-γ. Subsequently, the clinical efficacy of the treatment in all children 2 years after the treatment was analyzed.

Statistical analysis

Statistical significance was evaluated with SPSS 22.0 (IBM, USA). Data was expressed as mean ± SD and one-way analysis of variance was applied. $P < 0.05$ was considered significant.

RESULTS

T cell subset ratio

The proportion of $CD4^+$ $CD25^+$ T cells among $CD4^+$ T cells was not different before and after specific immunotherapy ($P>0.05$); the ratio of $CD4^+$ $CD25^{high}$ T cells in $CD4^+$ T significantly increased in 1 year and 2 years compared to the time before treatment ($P < 0.05$). However, the value 1 year of treatment was not significantly different from that of 2 years post-treatment (Table 1).

Changes in relative expression of FOXP3

The FOXP3 expression was enhanced 1 year after treatment compared to before treatment

Table 1: $CD4^+CD25^+/CD4^+$、$CD4^+CD25^{high}/CD4^+$ before and after specific immunotherapy

Groups	$CD4^+CD25^+/CD4^+$	$CD4^+CD25^{high}/CD4^+$
Before treatment	9.78±2.14	0.64±0.26
1 year after treatment	10.47±2.61	1.21±0.44*
2 year after treatment	11.09±2.47	1.20±0.51*
F	1.69	55.968
P	0.189	<0.001

**P*<0.05 (compared with those before treatment)

Table 2: Relative expression of FOXP3 before and after specific immunotherapy

Groups	Mean ΔCt value	$2^{-\Delta ct}$
Before treatment	8.74	1
1 yr after treatment	5.43	9.92
2 yr after treatment	7.09	3.14

Relative expression of IL-4, IFN-γ mRNA and ratio of IFN-γ/IL-4 before and after treatment and expression of IFN-γ and IL-4 in plasma

After 1 year of treatment, the relative expression of IFN-γ was significantly higher than that before treatment. After 2 years of treatment, the expression level returned to pre-treatment. The relative expression of IL-4 decreased with time; IFN-γ/IL-4 ratio increased significantly after 1 year of treatment. After 2 years of treatment, the rate decreased as compared to that a year before, and higher than that before treatment (Table 3).

The concentration levels of IFN-γ in the children increased significantly with time after treatment (P<0.05), and became stable after 1 year to 2 years but the concentration of IL-4 significantly decreased (P < 0.05), and equally became stable after 1 year to 2 years (Table 4).

(P<0.05) but decreased after 2 years post treatment even though it was still higher than that before treatment. The 2-ΔΔct value before treatment was taken to be 1 (Table 2).

Specific immunotherapy effectiveness

After 2 years for children with asthma, the rate of effectiveness of the specific immunotherapy was 87.72%. out of which the therapy was markedly effective in 66.67% of the patients.

Table 4: Levels of IFN-γand IL-4 in plasma (n=57)

Groups	IFN-γ (pg/ml)	IL-4 (pg/ml)
Before treatment	21.48±24.36	14.76±8.94
1 year after treatment	258.84±54.38*	6.47±5.12*
2 year after treatment	276.89±39.78*	5.09±4.31*
F	1761.358	127.203
P	<0.001	<0.001

**P*<0.05 (compared with those before treatment)

DISCUSSION

The etiology of allergic asthma is complex, the factors involved are complicated, and its pathogenesis has not been completely determined [7]. The industry attributes allergic asthma to imbalanced TH1/TH2 theory. For the treatment of allergic asthma, the traditional method adopts approaches like avoiding allergen exposure combined with local anti-inflammatory and antispasmodic drugs. The therapeutic action of such method is clear, but the side effects are severe, and it is likely to recur after drug withdrawal with short-lived effect, thus inducing drug dependence, and losing its efficacy as the dosage increases with time[8]. Specific immunotherapy is a treatment procedure by increasing or inhibiting the immune function of the body in response to the state of hypoimmunity or immune hyperfunction. This method is developed for the sake of prevention and radical cure. The patient can often prevent the recurrence of such diseases after long-term adaptive treatment, and improve airway and lung function. Studies have shown that specific immunotherapy has good curative effect on

Table 3: Relative expression of IFN-γ, IL-4 mRNA and IFN-γ/IL-4 before and after treatment

Groups	IFN-γ		IL-4		IFN-γ/IL-4 ratio
	Mean ΔCt value	$2^{-\Delta ct}$	Mean ΔCt value	$2^{-\Delta ct}$	
Before treatment	8.02	1	12.86	1	1
1 year after treatment	5.74	4.86	13.59	0.6	8.03
2 year after treatment	7.98	1.03	14.41	0.34	3.01

allergic rhinitis and asthma, and it can prevent and reduce the occurrence of rhinitis and asthma in early patients, but its precise mechanism and pathogenesis of asthma need to be further explored. $CD4^+$ $CD25^+$ T cells, FOXP3, IFN-γ, IL-4 play a pivotal role in asthma[9]. T cells are classified into $CD8^+$ and $CD4^+$ T subsets according to different cellular surface markers, and $CD4^+$ T cells can be further subtyped into TH1 and TH2 cells[10]. TH1 cells can secrete cytokines such as IFN-γ, which are mainly involved in cytotoxic and local inflammation-related immune responses, also known as inflammatory T cells. IFN-γ is a highly active multifunctional small molecule peptide that is an anti-asthmatic factor. It inhibits the synthesis of IL-4, and also the type conversion of inducing factors for IL-4 and IL-2, thereby inhibiting the production of IgE. TH2 cells mainly secrete cytokines such as IL-4. TH2 cytokines can induce macrophage and mast cell growth, differentiation and the formation of immunoglobulins such as IgE, participate in the pathogenesis of asthma, and lead type I hypersensitivity. IL-4 is a specific factor produced by TH2 cells and plays an important role in asthma-associated cytokines. TH1 and TH2 achieve TH1/TH2 balance through antagonizing each other. Related studies have pointed out that specific immunotherapy can transform $CD4^+$ T cells into TH1 type and promote the cytokine production including IFN-γ to correct the differentiation of TH1/TH2 cells and achieve a balance [11].

In recent years, $CD4^+$ $CD25^+$ T cells are engaged in the pathogenesis of AA. As a subtype of T cell, it is a hotspot of research [12]. Recent studies have found that FOXP3 is important for the function of $CD4^+$ $CD25^+$ T cells and is a molecular marker for it [13]. The experiment confirmed that mice FOXP3 mRNA and its encoded protein were uniquely expressed with $CD4^+$ $CD25^+$ T cells, indicating that FOXP3 gene takes part in obtain regulatory function of T cell [14].

This study found the proportion of $CD4^+$ $CD25^+$ T cells in $CD4^+$ T cells was not different in patients before and after specific immunotherapy ($P > 0.05$); compared with before treatment, the ratio after 1 year and 2 years of treatment, was significantly increased ($P<0.05$), and the $CD4^+CD25^{high}/CD4^+$ value was more stable 2 years after treatment as compared to that 1 year after treatment. It indicated that $CD4^+$ $CD25^{high}$ T cells increased with time extended, revealing that $CD4^+$ $CD25^{high}$ T cells are involved in alleviating asthma. After 1 year of treatment, the relative expression of FOXP3 was significantly higher than that before treatment ($P<0.05$). After 2 years of treatment, it slightly decreased than that after 1 year, but still higher than that before treatment. This indicated that elevated levels of FOXP3 expression during specific immunization promoted the inhibitory function of $CD4^+$ $CD25^+$ T cells to inflammatory cells and inhibited the development of asthma. FOXP3 plays an important inhibitory role in the treatment of allergic asthma in children with specific immunotherapy. 1 year post treatment, the relative expression of IFN-γ was significantly higher than that before treatment. After 2 years of treatment, the expression level returned to pre-treatment; the relative expression of IL-4 decreased with the prolongation of therapy; the rate of IFN-γ/IL-4 before treatment increased significantly. After 2 years of treatment, the ratio was lower than that a year before yet higher than before treatment. At the same time, the expression levels of IFN-γ and IL-4 in the children increased significantly as the treatment time extended, and the concentration became stable at 1 to 2 years after treatment. It is proved that the ratio of IFN-γ/IL-4 in the patient receiving the specific immunotherapy gradually increased over time, indicating that the imbalance of TH1/TH2 was gradually lessened and further stabilized to reach balance.

CONCLUSION

This study has revealed that *i*n the process of specific immunotherapy for children with allergic asthma, T lymphocyte subsets, FOXP3, IFN-γ, and IL-4 have significant roles even though FOXP3 factor is the key factor. FOXP3 can promote the expression of $CD4^+$ $CD25^+$ T cells, thereby regulating the expression of IFN-γ and IL-4 in patients, and promoting the balance of TH1/TH2. However, the mechanism of action is unclear and further studies to explore this is needed.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the manuscript for publication.

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