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The Effect of Immunotherapy on Bronchial Hyperresponsiveness in Asthmatic Children

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SUMMARY **Bronchial hyperresponsiveness (BHR) to methacholine were evaluated in 47 asthmatic children before and after allergen-specific immunotherapy (IT) by using the forced oscillation method. Eighty-seven percent (13/16) of BHR-negative patients had good clinical response after 1-year immunotherapy while there were only 45% (14/31) in the BHR-positive asthmatic children ($p < 0.02$). In the BHR-positive group, the relationship between clinical response and the change of non-specific bronchial sensitivity was further analyzed. In those of good clinical response (IT responder), the tolerance dose of methacholine was significantly increased from 0.78 ±0.71 to 4.11 ±4.65 mg/ml ($p < 0.05$), and bronchial sensitivity increased from 1.14 ±1.42 U to 7.55 ±9.55 U ($p < 0.02$). In those with no clinical improvement (IT non-responder), there were no significant changes in either methacholine tolerance dose or bronchial sensitivity. With respect to other parameters, such as Grs, PD_{35}, and SGrs, the differences between before and after immunotherapy were similar in both the IT responders and IT non-responders. These results suggest that asthmatic children with different bronchial sensitivity had different responses to immunotherapy and the clinical improvement after immunotherapy is significantly related to the improvement of bronchial hyperresponsiveness.**

Bronchial hyperresponsiveness (BHR) is one of the cardinal features of asthma,[1] and it has earlier been shown that the bronchial responsiveness to different stimuli are closely related in asthmatics.[2,3] BHR can be measured by either direct or indirect methods and the most commonly used measures are the methacholine challenge and exercise challenge tests.[4,5] The pathogenesis of BHR is complex and could involve a multiplicity of interactions between cellular components of inflammation, cytokines released, the destruction of airway epithelium and the stimulation of airway nerve endings.[6] Amelioration of bronchial hyperreactivity will result in improvement of symptoms and decrease of drug consumption.

It is generally recognized that 80% of childhood asthmatics and approximately 40% of adult asthmatics are allergic.[7] The exact relationships between allergy and asthma, or allergy and BHR are complex, and have not been completely understood.[8] Studies in children have shown that odds ratios for the association between increased airway responsiveness and skin test reactivity ranged between 1.5 and 9.2.[9-11] In these studies, the severity of allergy has been found to correlate with the degree of increased BHR. But whether the decrease of major allergen sensitivity by specific allergen immunotherapy (IT, or hyposensitization) will ameliorate BHR in atopic asthmatic children is a question which remains to be answered.

Despite its use in treating allergic disease for nearly eighty years, the efficacy of specific immunotherapy in bronchial asthma remains controversial.[12,13] Bronchial sensitivity in patients treated with immunotherapy has not been well studied in the past. Ohman *et*

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al[14] and Leng *et al*[15] have shown a decrease in bronchial sensitivity to cat allergen and pollen, respectively, after IT but only a few trials have used biologically standardized mite extracts as the antigen for immunotherapy[16,17] We have previously performed the methacholine inhalation challenge test in asthmatic children by using the forced oscillation method (Astograph TCK-6100, CHEST, Japan), and found that there was a close relationship between the level of increased non-specific BHR and the clinical severity of asthma in children.[18] The aim of the present study was to assess the bronchial response to methacholine in mite *(Dermatophagoides farinae;* D.f.)-sensitive asthmatic children before and after specific-allergen immunotherapy by using the forced oscillation method, and to evaluate the clinical response to allergen-specific immunotherapy in asthmatic children with or without bronchial hypersensitivity.

MATERIAL AND METHODS

Study populations

This study was an open, non-controlled trial of allergen-specific immunotherapy which was approved by the Human Research Committee of National Cheng-Kung University Hospital, and informed consents were obtained from all study subjects. The study population included 60 asthmatic children. The characteristics of the study populations are shown in Table 1. All the patients were sensitive only to mite *(Dermatophagoides farinae,* D.f.) in terms of a positive history of exposure, a >2+ skin test (wheal, >10 mm by prick test), and a positive RAST (Pharmacia Diagnostics AB, Uppsala, Sweden). IT was started with weekly injections of lyophilized allergenic extract of *D. farinae* (Pharmalgen standardized quality units [SQ], Pharmacia, Uppsala, Sweden), with known biological potency after reconstitution of 100,000 SQ/ml. This corresponded to 23,000 IU/ml, containing 10 μg/ml of the major antigen (D.f.I). The injection dose was increased gradually until a maximal tolerated dose was reached (usually within 6 months) and then was maintained at 4-week intervals. The total dose of crude mite allegen administered ranged from 1,260,000 to 2,190,000 SQ. The effectiveness of hyposensitization was evaluated by comparing both the frequency of asthmatic attacks and the amount of medications consumed after 1 year of treatment with those in the year before treatment. In this study, good responders consisted of those patients demonstrating an improvement of > 75% decrease of both parameters.[19] Among 60 asthmatic children enrolled in this study, 13 dropped out due to moving into other districts; 47 cases completed the course of therapy.

Bronchial provocation test

Details of this test has been fully described elsewhere.[18] In brief, bronchial provocation tests were carried out with an Astograph (TCK-6100, CHEST, Japan), which housed 12 nebulizers. Nebulizers No. 2-11 contained 3 ml of methacholine chloride solution (Daichi Pure Chemicals, Co, Ltd, Tokyo, Japan) in stepwise increasing concentrations, ie 0.048, 0.098, 0.19, 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, and 25.0 mg/ml, respectively. Nebulizer No. 12 contained 3 ml of 2.5 mg/ml of terbutaline as the bronchodilator for relieving bronchospasm. The nebulizers were driven by a constant air flow of 5 l/minute from the air compressor of the apparatus. The subjects were tested in a seated position with nose clip and were instructed to breath normally. Their cheeks were compress by a balloon to minimize oral pressure. All examinations were performed between 1 and 4 pm to avoid changes due to circadian rhythm of pulmonary function. The nebulizers were then actuated in sequence beginning with No. 1 (one minute for each nebulizer). Respiratory resistance (Rrs) was directly recorded by an X-Y recorder (Graphtec WX-2400). When the Rrs increased to twice the baseline value, or patients showed symptoms of intolerance such as difficult breathing or chest tightness, the test was interrupted immediately and terbutaline was inhaled. Nebulization was continued to the last concentration (25.0 mg/ml) of methacholine if there was no apparent change in Rrs.

Table 1. The clinical response to immunotherapy in BHR-positive and BHR-negative asthmatic children.

	BHR-positive (n=31)		BHR-negative (n=16)	
	IT responder	IT non-responder	IT responder	IT non-responder
Number	14	17	13	3
Age	10.2±2.3*	9.7±3.1	9.0±2.7	9.5±2.6
Sex (M : F)	9 : 5	10 : 7	11 : 5	2 : 1
Height (cm)	131.2±10.5	129.6±11.3	128.5±12.1	130.9±5.6
IgE (IU)	1,157±132	1,169±156	1,098±132	1.059±143

*Mean ± SD

Takishima *et al*[20] defined the subjects who had a bronchial sensitivity (Dmin) of more than 50 U (or log Dmin >1.699 log unit) as BHR-negative and the subject who had a Dmin of less than 50 U as BHR-positive. In BHR-positive patients, bronchial sensitivity was defined by the cummulative dose of methacholine required to provoke a positive reaction. Since Dmin is dependent on the flow rate and time of nebulization, it is best expressed in methacholine units. One units is equal to one minute of inhalation of aerosol solution at 1.0 mg/ml of methacholine during quiet tidal breathing.[2] According to our previous experience in the study of bronchial responses of school children,[21] a high proportion of nonasthmatic subjects developed bronchial constriction after inhalation of 6.25 mg/ml or higher of methacholine (ie, Rrs increased 2 times before inhalation of nebulizer No. 7 [methacholine 6.25 mg/ml]). Therefore, this unit was chosen as the cut off point for BHR. The respiratory conductance (Grs) was calculated from the reciprocal of Rrs (1/Rrs). Because the slope of Grs (SGrs = Grs/t) in a positive reaction is more linear than that of Rrs, SGrs (in l/sec/cm H_2O/min.) is defined as the bronchial reactivity. The bronchial responsiveness was expressed as the cumulative dose of methacholine required to produce a 35% decrease in SGrs ($PD_{35}SGrs$). Difference of astographic parameters before and after immunotherapy were analyzed for their correlation with clinical responses.

Statistical analysis

Difference of clinical responses between groups of BHR-positive and BHR-negative was analyzed by chi-square test. Statistical difference between the data of astographic examination before and after immunotherapy were analyzed by unpaired *t* test. Statistics were computed using the SAS (statistical analysis system) for personal computers.

RESULTS

Forty-seven asthmatic children who had completed the full course of immunotherapy were included in the final analysis. These patients were further grouped according to the result of astographic examination before immunotherapy as: BHR-positive (n = 31, ie Rrs increaed twice before inhalation of a cumulative dose of methacholine of 6.25 mg/ml) and BHR-negative (n = 16, ie Rrs increased twice at and after inhalation of a cumulative dose of methacholine of 6.25 mg/ml). After one year course of conventional high dose immunotherapy and compared to the clinical symptoms before treatment, there were 14 IT responders and 17 IT non-responders in the BHR-positive group. On the contrary, there were 13 IT responders and 3 IT non-responders in the BHR-negative group (Table 1). The response to immunotherapy was significantly different between BHR-positive and BHR-negative group ($p<0.002$; by chi-square test).

Thirty-one BHR positive patients were further analyzed for the relationship between improvement of BHR and the response to immunotherapy. Methacholine tolerance dose (Cmin) were increased from 0.78 ± 0.71 to 4.11 ± 4.65 mg/ml in IT-responders after one year of conventional high dose of immunotheapy ($p < 0.05$). Bronchial sensitivity (Dmin) had also increased from 1.14 ± 1.42 U to 7.55 ± 9.55 U in the IT-responders ($p < 0.02$). There were no significant changes in methacholine challenge dose or

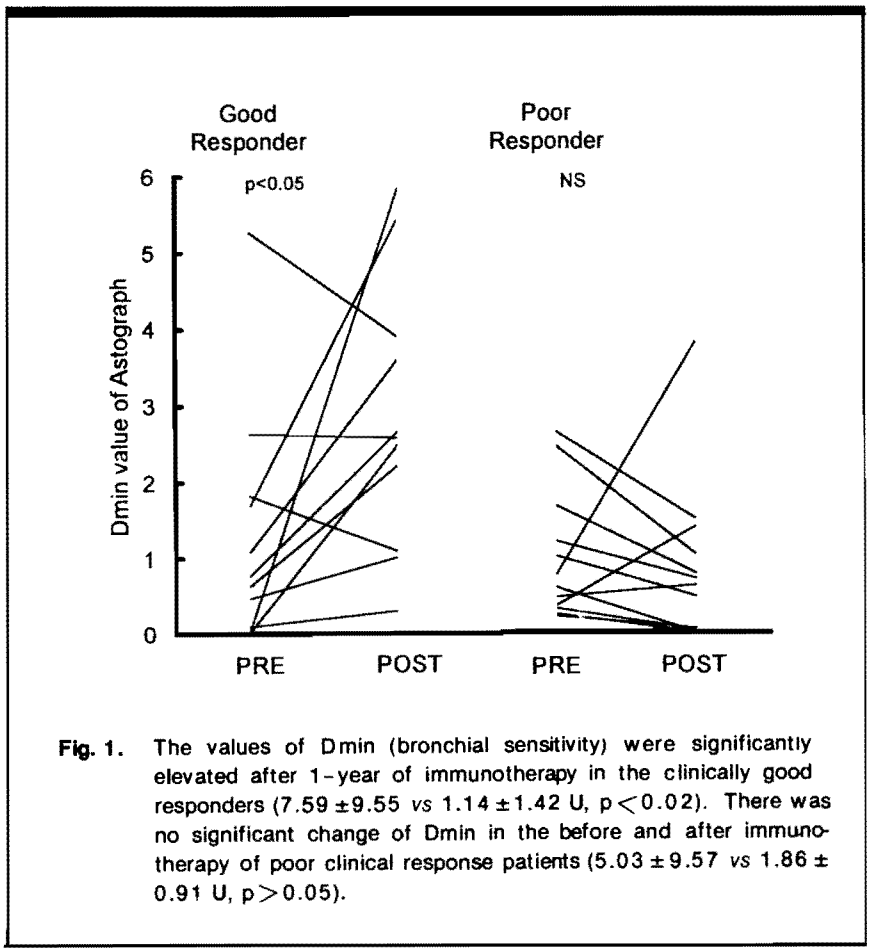

Fig. 1. The values of Dmin (bronchial sensitivity) were significantly elevated after 1-year of immunotherapy in the clinically good responders (7.59 ± 9.55 *vs* 1.14 ± 1.42 U, $p<0.02$). There was no significant change of Dmin in the before and after immunotherapy of poor clinical response patients (5.03 ± 9.57 *vs* 1.86 ± 0.91 U, $p>0.05$).

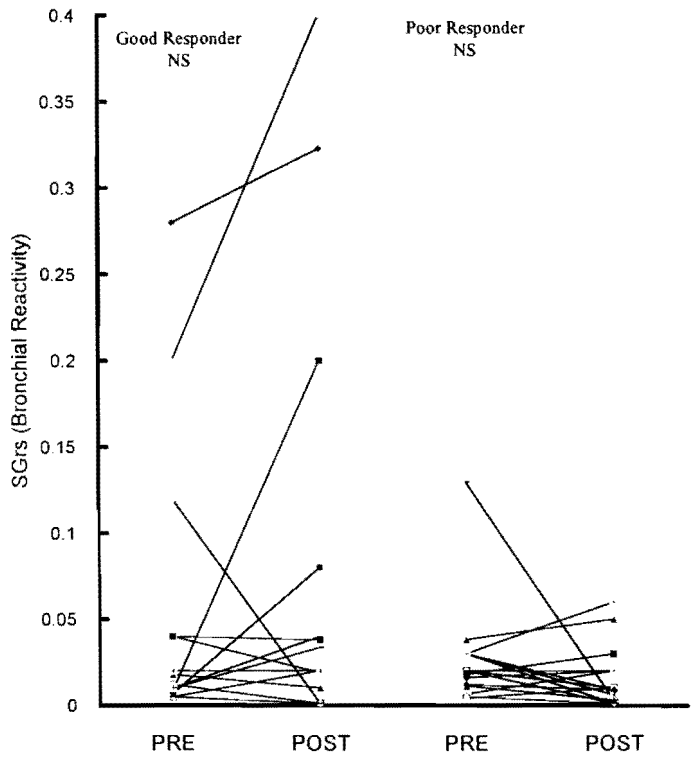

Fig. 2. The difference of bronchial reactivity (SGrs) between before and after immunotherapy were not significant in the IT responders and IT non-responders.

Table 2. The astographic parameters in BHR-positive asthmatic children before and after immunotherapy.

	Clinical response	Mean ± SD	
		Before IT	After IT
Dmin (U)	poor (n=14)*	1.86 ±0.91	5.03 ±9.57
	good (n=17)	1.14 ±1.42	7.59 ±9.55
Cmin (mg/ml)	poor	0.58 ±0.57	2.67 ±2.25
	good*	0.78 ±0.71	4.11 ±4.65
Grs (l/sec/cmH_2O)	poor	0.15 ±0.03	0.17 ±0.04
	good	0.14 ±0.03	0.17 ±0.04
PD_{35} (U)	poor	11.7 ±9.91	14.74 ±10.89
	good	14.62 ±9.66	16.89 ±8.21
SGrs (l/sec/cmH_2O/min)	poor	0.025 ±0.028	0.015 ±0.017
	good	0.016 ±0.011	0.025 ±0.021

*Significant difference ($p < 0.02$) of Dmin between before and after immunotherapy

bronchial sensitivity in IT non-responders after immunotherapy (Fig. 1). Regarding the other parameters, such as Grs, PD_{35}, and SGrs, the differences between before and after immunotherapy were not significant in both the IT responders and IT non-responders (Table 2, Fig. 2).

DISCUSSION

Although the bronchial hyperresponsiveness (BHR) is one of the etiologic factors in bronchial asthma, the exact cause of BHR in asthma is uncertain. Previous studies have reported that BHR is induced by respiratory exposure to ozone, infection, air pollution and antigen provocation, however, the increase in reactivity does not last very long, while the BHR in asthma is more stable and persistent.[21] Cockroft and co-workers[22] have examined determinants of allergen-induced asthma in atopic asthmatic children. They found that underlying bronchial responsiveness to allergen was a function of three factors: firstly,the dose of allergen to which the individual is exposed; secondly, the level of circulating IgE antibody to that allergen, and thirdly, the underlying degree of nonspecific bronchial responsiveness. Our previous study has shown that there was a close relationship between the nonspecific BHR and clinical severity of asthmatic children.[18] This may be explained by the facts that repeated allergen exposure and airway inflammation may result in both worsening BHR and increasing clinical severity.

Despite its use in the treatment of allergic disease for nearly eighty years, the efficacy of allergen-specific immunotherapy in bronchial asthma remains controversial. We have previously reported that the allergen-specific IT was able to reverse the abnormal production pattern of inflammatory mediators

and cytokines (ie histamine, leukotriene C4, prostaglandin E2, interleukin-1, and tumor necrosis factor) in IT responder asthmatic children.[23,24] It is reasonable to suspect that allergen-specific IT may improve the BHR status in asthmatic children via the improvement of chronic inflammation in the airways. In this study, we have found that BHR-negative asthmatic children were more likely to have a good clinical response after one year of conventional high dose of immunotherapy. We also found that the improvement of bronchial sensitivity (Dmin) and tolerance dose to methacholine (Cmin) after immunotherapy was closely related to the clinical benefit evaluated by records of asthmatic attack and medications consumed.

Heterogeneity of clinical severity in asthmatic children has been noted for a long time, whereas few studies were designed to analyze the difference between these groups. We have previously shown that the bronchial sensitivity (Dmin of astographic examination) were closely related to the clinical severity in childhood bronchial asthma.[18] In this study, we found that asthmatic children with different bronchial sensitivity had different responses to immunotherapy. Patients with low initial Dmin who had good clinical responses after IT can be interpreted as having mild asthma which resulted in the most improvement. These results are in agreement with those of Bousquet *et al*[25] who reported improvement after immunotherapy to be significantly related to clinical severity.

Improvement of end-organ responsiveness after immunotherapy is an important clue to improvement. However, it is time-consuming to perform the standardized procedure of methacholine inhalation challenge in younger children. It has been performed only in a few studies related to immunotherapy of childhood bronchial asthma. Astograph may be an alternative to the stardard methacholine challenge test. Most importantly we have shown evidence that astographic examination may have important role in predicting the clinical response after immunotherapy. This grouping according to their bronchial sensitivity may be a useful model for analyzing the effect of immunotherapy.

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