Asian Pacific Journal of Allergy and Immunology (1994) 12 : 125-129

Airway Provocation Test with Ragweed Pollen Extract in Chinese Asthmatics

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SUMMARY As ragweed becomes widespread over China, ragweed pollenosis tends to be more frequent. Incidence of ragweed pollenosis is about 1.04% in Qingdao district. To elucidate ragweed as an allergen in the development of bronchial asthma, ragweed pollen extract inhalation airway provocation tests (APT) were studied in 55 patients with ragweed pollenosis. A single dosage of 9,000 PNU ragweed pollen extract was applied and positive response was defined as more than 35% fall in SGrs at the dose of PT35-Grs. Among 55 patients, positive APTs were found in 27 cases (49%), dual late-phase responses in 11 patients, single late-phase responses in 2 patients in the complicated asthma group. Late-phase responses were related to the increased specific IgE and strong positive skin test response (wheal >11mm). The results suggested ragweed pollen was one of the important allergens causing bronchial asthma. There was airway hyperresponsiveness to ragweed pollen in some patients (20%) with ragweed pollenosis without asthmatic symptoms.

As ragweed becomes widespread over China, ragweed pollenosis tends to be more frequent.[1,2] According to our investigation in 10,091 inhabitants, the incidence of ragweed pollenosis is about 1.04% in Qingdao district, Shangdong Province.

Bronchial asthma is an inflammatory reaction induced by allergens or other factors. After inhalation of allergens, it can provocate two responses, immediate response and late-phase response. In our data, many patients with ragweed pollenosis had complicated bronchial asthma. Therefore, a new airway provocation test was applied. The inhalation challenge with a single dosage of ragweed pollen extract was performed. According to series of measurements from lower concentrations increasing to 1:5 w/v in the provocation test, this allergen concentration was chosen.

Our studies suggested that ragweed pollen is one of the important allergens causing bronchial asthma and also showed late-phase asthmatic responses induced ragweed pollen extract were related to the increased IgE in the serum and strong positive skin test response.

MATERIALS AND METHODS

Subjects

Fifty-five patients with ragweed pollenosis (31 males and 24 females) with a mean age of 35.4 years (18-64 years) participated in the study. The ragweed pollen season was from August to October. All subjects received intradermal skin tests (ST) of ragweed pollen extract diluted 10^{-3} w/v. A positive response occurred in all of them. The intranasal provocation test using filter paper absorbed with ragweed pollen extract was performed.[3-5] The test extract content was about 450 PNU and positive response were obtained in all cases. Specific serum IgE using the BA-ELISA method was determined in 36 cases.[6,7] Among 55 patients, 25 cases had complicated asthma (group

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A) and 30 cases were asthmatic symptom free (group B). Twenty sex and age matched normal subjects without atopic history were selected as a control group, whose ST and intranasal provocation tests were negative.

Skin tests with allergen

The skin tests by intradermal injection were performed with ragweed *(Ambrosia artemisiifolia)* pollen extract diluted 10^{-3} w/v. The skin testing preparation of 0.02 ml was injected in the upper arm; at the same time, injection of other pollen allergen extracts such as Artemisia, Humulus, Grass, etc, and Coca control solution was carried out using the same method. The interval length between every two points of injection was 3 cm. The sizes of wheal and flare responses were measured at fifteen minutes after injection and late responses of ST were measured at 4 to 8 hours.

We identified positive skin testing results as a wheal of more than 5×5 mm; the mean wheal diameter was 5–10 mm. If the mean diameter of the wheal was over 11 mm, it was defined as a strong positive response. When its diameter was less than 5 mm, the result was defined as a negative response.

Specific serum IgE determination

Specific serum IgE was determined using the BA-ELISA. This method was established using the sensitive Avidin-Biotin system. Ragweed pollen extract was diluted at 1:20 in 0.1 M carbonate buffer (pH 9.6).

When the OD reading of the sample was greater than 2.1 times the negative reference reading, it was defined as positive IgE. When the OD reading of the sample was greater than 3.1 times the negative reference reading, it was defined as a strong positive IgE.

Measurement methods of APT

Airway responsiveness was measured (Astograph TCK-6100 H, Japan).[8-11] During 48 hours before measurement, antihistamine and corticosteroid drugs were not administered.

All chest x-ray films were normal and spirometer determined FEV_1 values were not less than 70% of the predicted value.

The measurement of immediate provocation response

The airway provocation test (APT) with inhalation of ragweed pollen extract was studied in 55 patients with ragweed pollenosis. A single dose of 9,000 PNU ragweed extract was applied. It was conducted in the remission stage of asthma for group A and in the ragweed pollen season for group B and the control group.

The procedure was as follows: The measurement of Rrs, c (baseline of respiratory resistance) was started. All subjects inhaled an aerosol of 1:5 w/v ragweed pollen extract for 1 minute (about 9,000 PNU) after it had been confirmed that a 1 minute inhalation of physiological saline did not change their baseline Rrs. Subjects began to inhale aerosolized 0.05% isoprenaline sulfate for 2 minutes, when their Rrs reached twice that of the baseline value within 10 minutes from the start of inhalation of ragweed pollen extract, or when subjects indicated signs of dyspnea after inhalation of the ragweed pollen extract, they also then inhaled aerosolized 0.05% isoprenaline sulfate for 2 minutes. All subjects' Rrs were restored to the baseline value during the observation, within 10 minutes after inhalation of the ragweed pollen extract.

Depending on the Rrs response curve following inhalation of the extract, we calculated four parameters: (1) Rrs. c: the resistance during inhalation of saline aerosol; (2) Ta: time period from the start of the extract inhaled to the beginning of Rrs increase, ie airway sensitivity; (3) SGrs: decreasing rate of conductance for per minute, ie airway reactivity; (4) PT35-Grs: the time from start of the extract exposure to the point at which Grs decreased 35% from baseline value. According to the data, we defined a positive response of APT as more than 35% fall SGrs at the dose of PT35-Grs.

The measurement of late-phase response (LPR)

After APT, Rrs was measured per hour, within 4 hours. The following procedure was conducted 2 times with an interval of two hours; the final time of measurement was 24 hours later. If the test subjects manifested dyspnea symptoms during provocation test, whether their responses were positive or negative, the Rrs was measured immediately. The late-phase response was defined as the continuing airway hyperresponse condition maintained more than 4 hours, or more than 35% fall in SGrs at that dose after 4 hours provocation test. The dual late-phase response was defined as the positive immediate response of APT following a late-phase response. A single or isolated late-phase response was defined as LPR only if it occurred without immediate positive APT.

RESULTS

In 55 patients with ragweed pollenosis, 27 cases (49%) had a positive APT; 21 cases were in group A and 6 cases were in group B. Positive APT was not found in the control group. Dual late-phase response occurred in 11 cases, of which 9 cases were in group A. Single late-phase response was only found in 2 cases of complicated asthma (group A). Late-phase responses occurred in all 13 cases, a positive rate of 23.6% with LPR of APT (Tables 1, 2 and Fig. 1).

Table 1. The results of APT with ragweed pollen extract.

Group	Rrs.c ($cmH_2O \cdot L^{-1} \cdot S^{-1}$)	Ta (min)	SGrs ($L \cdot S^{-1} cmH_2O^{-1} \cdot min^{-1}$)
A	4.5 ±2.2	3.5 ±2.2	0.04 ±0.02
B	4.2 ±1.5	4.8 ±2.6	0.02 ±0.01
Control	2.8 ±0.8	8.3 ±1.7	0.01 ±0.01

Table 2. Statistical test of patient groups and control group.

Comparison	Rrs.c		Ta		SGrs	
	t	*p*	*t*	*p*	*t*	*p*
Control group with group A	3.8	<0.01	2.9	<0.01	3.4	<0.01
Control group with group B	4.3	<0.01	5.7	<0.01	1.4	>0.05

Among 19 patients (52.6%) with positive specific IgE, 10 cases had late-phase responses. Those patients with marked positive IgE were easier to detect LPR than subjects with negative IgE ($p < 0.05$).

In 55 cases with ragweed pollenosis who received skin tests, a positive response (5–10 mm wheat) occurred in 21 cases and > 11 mm wheal in 34 cases. In 5 cases a wheal >11 mm was followed by late cutaneous responses (Tables 3, 4).

In 29 cases of positive APT, 23 cases had a wheal diameter of ST > 11 mm; compared with the cases with negative APT, the p value was less than 0.01. The wheal diameter of ST was >11 mm in 13 cases with LPR.

DISCUSSION

The airway responsiveness measurement using an inhalation method with a single dosage of high concentration ragweed pollen extract was

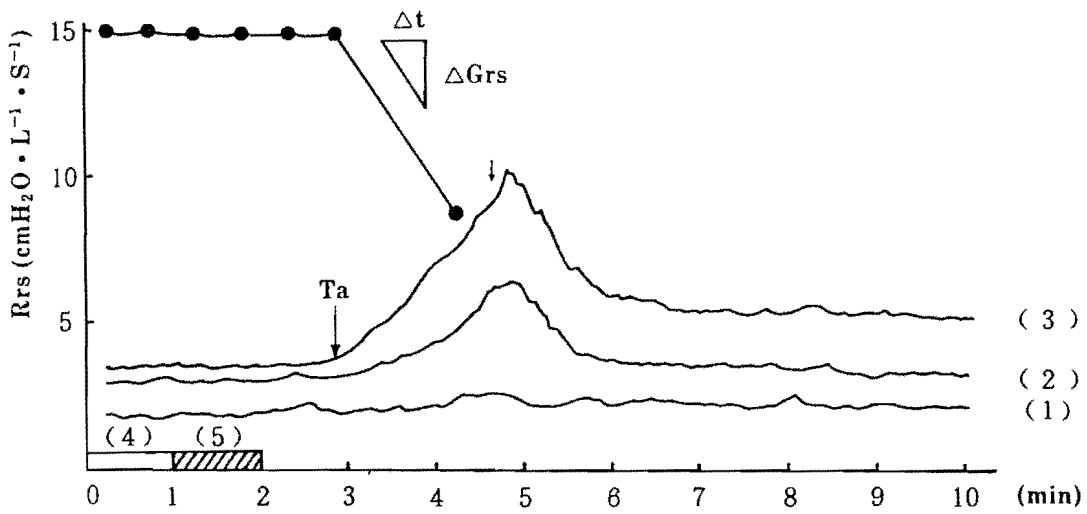

Fig. 1. The Rrs response curves during inhaling ragweed pollen extract. The three curves studied typical subjects of different groups are illustrated. (1), control group; (2), group B; (3), group A; (4), inhalation time of physiologic saline; (5), inhalation time of ragweed pollen extract. ↓ means inhalation of bronchodilator
$cmH_2O \cdot L^{-1} \cdot S^{-1}$ = cmH_2O/litre/sec.
Grs = 1/Rrs SGrs = △Grs/△ t.

Table 3. Skin test results.

Ragweed pollenosis	Wheal diameter (mm)	Statistical analysis	
		t	*p*
With asthma	12.72 ±3.96	2.009	> 0.05
Without asthma	10.87 ±4.06		

The 5 cases (wheal >11 mm) followed late cutaneous responses, in which 4 cases occurred in group with asthma.

Table 4. Correlation of ST and APT.

Wheal diameter (mm)		Positivity of APT			
		Immediate response		LPR	
Group A	Total	No.	%	No.	%
5-10	6	4	66.7	0	0
11-20	19	17	89.5	11	61.1
Total	25	21	84.0	11	44.0
Group B					
5-10	14	0	0	0	0
11-20	16	6	37.5	2	10.5
Total	30	6	20.0	2	6.7

In positive immediate response, comparison of wheal >11 mm group with wheal of 5 to 10 mm group obtained $x^2 = 10.64$, $p < 0.01$.

tested in 55 patients with ragweed pollenosis. Though the patients with complicated asthma received APT in the non-pollen season, many subjects also manifested airway hyperresponsiveness, the positive rate being 84%. There was airway hypersensitivity to ragweed pollens in some patients with ragweed pollenosis without asthmatic symptoms. Therefore, these patients should be followed up because they are likely to develop asthma in the future. The results suggested that ragweed pollen was one of the important allergens causing bronchial asthma in China.

In 29 patients with positive APT, 23 cases (79.3%) had ST wheal diameter >11 mm, which indicated that there was a significant relationship between APT and ST. Therefore, our results are in accord with data published by Holman *et al.*[12,13]

Our study demonstrated that late-phase responses were related to increased IgE and strong positive ST responses (wheal >11 mm).

Bronchial asthma during an attack can easily be diagnosed, but patients in remission stage may give essentially normal spirographic tests. Consequently, the measurement of airway responsiveness is very important in the diagnosis of asthma and in latent asthmatics in particular. The different spirometer measurements have been applied as in the conventional method of the bronchial provocation test.[14,15] The major testing indicator is FEV_1, yet, this examination requires forced expiratory efforts by patients, which may lead to release of histamine. On the other hand, examination with the astograph can be used during normal breathing, which is suitable for patients. Although the airway provocation test in this study is not a standard procedure currently used internationally, it is acceptable in some countries such as China and Japan.

The two responses, immediate and late-phase, usually result from exposure to allergen. In the past, widespread attention of investigators focused on the mechanism of LPR as induced by IgG. However, according to recent studies of many authors, it would be considered as an IgE-mediated reaction. IgE is even more important than IgG.[16-21] A lot of studies have confirmed that LPR is an allergic inflmmatory response due to activation of chemical mediators and various chemotactic factors such as leukotrienes, ECF, NCF and PAF released by mast cells, basophils, neutrophils, eosinophils, monocytes and macrophages. Furthermore, the mechanism causing LPR is related to other factors, including the properties and concentration of exposure allergen as well as certain non-specific inflammation reactions. Many investigators suggest that LPR is more similar to chronic asthma than to acute bronchospasm. LPR corresponds with the attack mode of bronchial asthma.[21,22] Therefore, the studies of LPR in asthma have important clinical value.

ACKNOWLEDGEMENTS

This study was supported by a grant from Qingdao Research

Institute of Integrated Chinese Traditional and Western Medicine. We are thankful to Miss Bi Yan for typing the manuscript.

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