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Bronchial Challenge Responses in Asthmatic Patients Sensitized to Artemisia Spp. Pollen

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To characterize the patients whose asthma may be caused by Artemisia pollen extracts, we studied the bronchoprovocation test with Korean Artemisia pollen extracts (1:20 w/v), methacholine bronchial challenge test and wormwood-RAST in 32 asthmatic patients sensitized to Artemisia pollen. Twenty-six(81%) developed a 15% or greater decrease in FEVI after the inhalation of Artemisia pollen extracts and 13 patients showed early responses, 8 dual, and 5 late only. Thirteen(50%) out of 26 positive responders complained of seasonal aggravation of their asthmatic symptoms. Seven(53.8%) of the 13 seasonal type patients, 10(76.9%) of the 13 perennial type and 5(100%) of the 5 negative responders showed concurrent positive responses in the house dust bronchoprovocation test. The bronchial responsiveness to allergen(PD15) was more dependent upon the specific IgE level(bound radioactivity on wormwood-RAST) and multiple regression analysis revealed that the specific IgE level and methacholine PC20 may be contributory to allergen PD15. These results suggested that specific IgE to Artemisia pollen appears to be the major contributor to susceptibility to Artemisia bronchial challenges and this pollen may be considered as one of the important allergenic etiologies of atopic asthma in this country.

Key Words: Artemisia spp. pollen, bronchial asthma, bronchoprovocation test.

The relationship between asthma and allergic responses to pollen allergens needs further clarification. Although house dust mites are the most important inhalant allergen in this country (Kang *et al.* 1973; Whang *et al.* 1974; Hong *et al.* 1982; Kim *et al.* 1983), the pollen of Artemisia is abundant in the air of Seoul from the end of August through September (Min *et al.* 1984; Hong *et al.* 1986) and is considered as the main late summer-autumn allergen source in respiratory allergic patients (Hong *et al.* 1981; Park *et al.* 1988). Many asthmatic patients who developed reversible airway obstruction after the inhalation of Artemisia pollen extracts showed concurrent positive results on the house dust-bronchoprovocation test and the exacerbation of their symptoms occurred without a marked seasonal pattern.

In this study, in order to better characterize the patients whose asthma may be induced by Artemisia pollen extracts, we compared historical, immunological and bronchial sensitivity in 32 asthmatic patients sensitized to Artemisia pollen.

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MATERIALS AND METHODS

Patients

Thirty two asthmatic patients with positive skin prick tests to Artemisia pollen were studied. Each patient was tested on the back with a 1:20 w/v solution of Artemisia spp. pollen extracts. The patient's serum was assayed for total IgE and specific IgE to Artemisia pollen. The history of each patient was obtained and seasonal variations of asthmatic symptoms were recorded.

Preparation of Korean Artemisia Pollen Extracts

Artemisia pollens were collected during their season. They were defatted with ethyl ether, dried and extracted with modified Coca's solution (NaCl 9gm, phenol crystal 4gm, NaHCO3 2.5gm in1000ml of distilled water) in 1:10 w/v at room temperature for 72 hours. The supernatant was dialyzed against an adequate amount of 0.4% phenol-0.9% NaCl and mixed with an equal amount of sterile glycerine to yield the 1:20 w/v solution of Korean Artemisia pollen

extracts for the skin test. For the antigen of the bronchoprovocation test, defatted Artemisia pollens were extracted with modified Coca's solution in 1:20 w/v at room temperature and centrifuged, and were dialysed against 0.4% phenolized saline, stored in a small sealed vial at −20°C and used as an antigen on the day of the bronchoprovocation test.

Allergy Skin Test

Skin prick tests with Korean pollen extracts, and Bencard's (Great Britain) and Torii's (Japan) common inhalant allergen extracts were performed simultaneously. The results were read 15 minutes after the prick. The wheal and erythema size were measured as maximum diameter and vertical length at the mid-portion of maxmum length and presented as the ratio of allergen and histamine reaction (A/H ratio).

Methacholine Bronchial Challenge Test

Nonspecific bronchial reactivity was determined by the previously described standard method (Chai *et al.* 1975). An aerosol of 0.9% NaCl followed by doubled concentrations of methacholine (0.075 to 25.0 mg/ml) was inhaled. The forced expiratory volume (FEVI) was measured 5 minutes after each inhalation and continued until the FEVI had fallen by 20% (calculated from the post-saline value). The provocation concentration of methacholine required to reduce FEVI 20% below baseline(PC20) was obtained from the dose response curve.

Bronchoprovocation Tests with Allergens

Bronchoprovocation tests were performed, using aqueous extracts of Korean Artemisia pollen (1:20 w/v) and house dust (1:10 w/v, Torii Co.) by the modified method of Chai *et al* (1975). The FEVI and maximum mid-expiratory flow (MMEF) were measured with a spirometer before inhalation and 10 minutes after inhalation. The test solutions were delivered by a Vaponefrine nebulizer and compressed air source and the patients were asked to breathe the nebulized aerosol 5 times until their vital capacity was achieved. A 0.4% phenolized saline solution was inhaled for a baseline value and serial increments in antigen concentration (1:500, 1:100, 1:50, 1:20 w/v) were given at 10 min intervals until a 20% or greater decrease in FEVI from the baseline value was recorded. If there was no significant change in pulmonary function test after the inhalation of the highest antigen concentration, the patients were asked to inhale 1:20 w/v of Artemisia antigen once more. The pulmonary function test was performed frequently during the first hour, and then the FEVI and MMEF were measured at hourly intervals for 7 hours after the challenge. One breath of 1:50 w/v Artemisia antigen was decided as 1 arbitrary unit and the cummulative dose of inhaled Artemisia antigen required to produce a 15% decrease in FEVI was obtained for each patient(PD15).

Specific IgE Antibodies to Artemisia Pollen and Common Inhalant Allergens

IgE antibodies to Artemisia pollen were assayed by the wormwood-radioallergosorbent test (RAST) using the commercial paper disk method (Pharmacia Diagnostics, Uppsala, Sweden), and those to other inhalant allergens were also assayed by RAST. The commercial kit was used as indicated by the manufacturer. The results were expressed as the percent count bound divided by total count added (% B/T) and RAST class as proposed by the manufacturer.

Statistics

The means of the groups were compared by the student's t-test. Multiple regression analysis on allergen PD15 was performed using three independent variables, methacholine PC20, RAST, and skin reactivity. Of these variables, allergen PD15 and methacholine PC20 were logarithmically transformed.

Table 1. Baseline pulmonary function test (PFT) of challenged patients

Asthmatic responses / PFT	Negative (n=6)	Early (n=13)	Dual (n=8)	Late (n=5)
FVC (%)	92.9±10.34*	95.3±17.58	106.1±10.08*	94.1±11.11
FEV1 (%)	70.4±14.03*	78.4±18.63	83.7±9.00+*	66.4±14.51+
MMEF(%)	53.0±21.38	61.7±25.51	55.1±10.25	42.6±18.56

* : $p<0.05$, Compared between negative and dual responders

+: $p<0.05$, Compared between dual and late only responders

RESULTS

Patients' Characteristics and their Pulmonary Function Test

Of the 32 asthmatic patients who had positive skin tests to Artemisia pollen extracts, 26(81%) developed a 15% or greater decrease in their FEVI after the inhalation of Artemisia pollen extracts (13 early, 8 dual, and 5 isolated late responses). Their initial pulmonary function tests showed that there were no significant differences in FEVI between negative and isolated late responders, as shown in Table 1.

Ten out of the 26(38.5%) patients who had positive challenges developed their asthmatic symptoms throughout the year, whereas only 7(26.9%) patients complained of seasonal exacerbations. As the duration of study of 9 patients was less than 1 year, their seasonal variations could not be determined definitely. Among them, 6(23.1%) patients were noted to develop their asthmatic symptoms just after the autumn season and the 3 patients did not (Table 2).

Table 2. Seasonal variation of asthmatic attack

	Positive responder (n=26)	Negative responder (n=6)
Perennial	10*	3
Seasonal*	7	3
Undetermined (<1yr)	9	0
in season	6	
not in season	3	

+ Artemisia Pollen Season: August - October
* One patient: worse form June, and another one; worse in spring and fall

Skin Prick Test and RAST

As shown in Table 3, skin test response and IgE antibodies to Artemisia pollen were higher in the group with positive challenges, whether they were the perennial or seasonal type, as compared to those with negative bronchial challenge tests, but the skin test response to house dust and house dust mites was

Table 3. Comparison of skin prick test and RAST results of challenged patients

Allergen	Positive responder (n=26)				Negative Responder (n=6)	
	Perennial type* (n=13)		Seasonal type** (n=13)			
	Skin test† (≥2+)	RAST††	Skin test (≥2+)	RAST	Skin test (≥2+)	RAST
Alder	3	1/2 (50%)	4	2/7 (29%)	0	0/1
Oak	6	1/4 (25%)	3	2/3 (67%)	0	0/1
Popula	7	0/2 (0%)	6	0/2 (0%)	1	0/2
Bermuda	3	0/6 (0%)	2	3/6 (50%)	1	0/2
Timothy	3	0/1 (0%)	1	2/2 (100%)	0	–
Ragweed	6	2/9 (22%)	8	5/10 (50%)	0	0/3
Wormwood	9	8/13(62%)	11	11/13 (85%)	3	1/5 (20%)
Korean Artemisia spp.	13	ND	12	ND	4	ND
Penicillium	4	0/2 (0%)	5	0/3 (0%)	2	0/1
Alternaria	5	0/3 (0%)	5	1/3 (33%)	3	0/1
Aspergillus	5	0/7 (0%)	4	0/5 (0%)	0	0/1
Cat	10	2/9 (22%)	9	2/6 (33%)	5	2/5 (40%)
House dust	13	7/13(54%)	12	8/11 (73%)	5	3/5 (60%)
D. pteronyssinus	11	9/13(69%)	10	10/12 (83%)	5	5/5(100%)
D. farinae	10	9/13(69%)	10	10/12 (83%)	5	5/5(100%)

* Perennial : perennial cases (10) + undetermined cases (3) developing asthma in non-seasonal months
** Seasonal : seasonal cases (7) + undetermined cases (6) developing asthma in seasonal months
† Skin test : number of positive skin prick tests (≥21 mm of erythema)
†† RAST : number of positive RAST results/number of RAST tested

Table 4. Results of bronchoprovocation test with house dust and other pollens in Artemisia positive responders

	Seasonal	Nonseasonal
House dust positive	7	10
House dust dominant	3	5
Artemisia dominant	4	5
House dust negative	6*	2
Other	0	1**
Total	13	13

* One case: positive to ragweed challenge test

** Positive to hop Japanese challenge test (not tested with house dust)

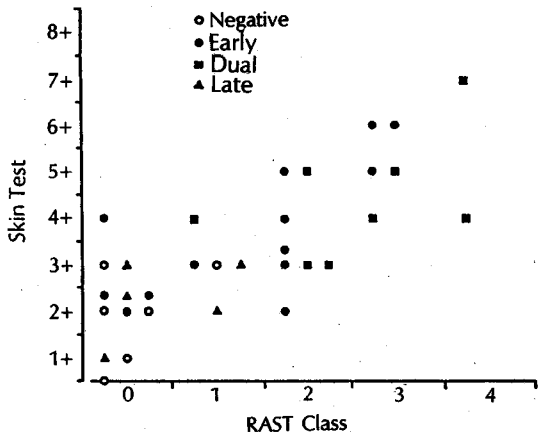

Fig. 1. The relationship among skin reactivity (A/H ratio) to Korean Artemisia pollen extracts, wormwood-RAST and bronchial challenge responses.

similar between the two groups.

Bronchoprovocation Test with House Dust and Other Pollens.

Seven(53.8%) out of the 13 seasonal type patients, 10(76.9%) of the 13 non-seasonal type and 5(100%) of the 5 negative responders showed concurrent positive results on the house dust bronchoprovocation test. Among 6 cases of negative responders, the house dust bronchoprovocation test was not performed in one case of the non-seasonal type, as this patient showed negative response to house dust mites on the skin prick test(Table 4).

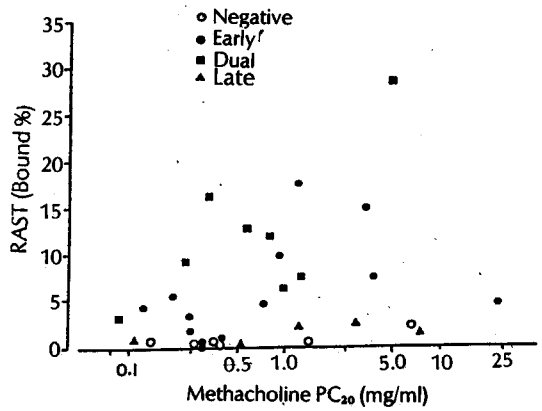

Fig. 2. The relationship between PC20 of methacholine and wormwood-specific IgE level as % bound radioactivity.

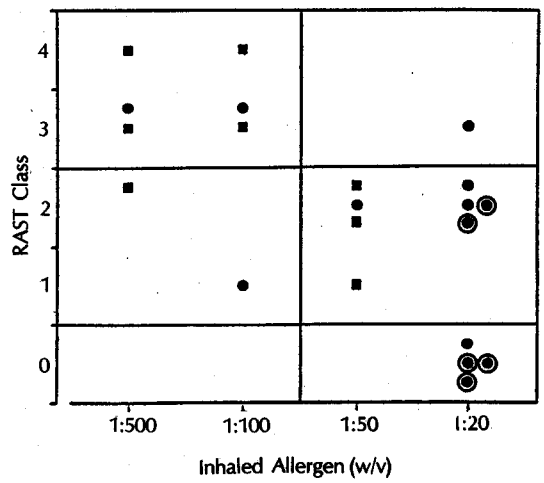

Fig. 3. The relationship between bronchial threshold to Artemisia antigen and wormwood-RAST class in early (●) and dual (■) responders. Five early responders were asked to inhale 1:20 W/V of Artemisia antigen two times (◉)

The Relationship Among Skin Reactivity to Korean Artemisia Pollen Extracts, Wormwood-RAST and Bronchial Challenge Responses.

Seven(63.6%) out of 11 patients who had wormwood-RAST class 0 showed an early or isolated late response after inhalation of Artemisia pollen extracts. All who had wormwood-RAST class 2 or higher showed a positive bronchial challenge response. As the RAST class increased, dual asthmatic responses increased as shown in Fig. 1.

Table 5. Regression analysis on PD_{15} of allergen

Independent variables	R-square	Adjusted R-square	F-value
Wormwood-RAST (Bound radioactivity)	0.4412	0.4118	15.0028**
Log_{10} Methacholine PC_{20} (mg/ml)	0.0002		
Skin reactivity (A/H ratio)	0.2729	0.2346	7.1294*
RAST+Log_{10} mathacholine PC_{20}	0.5357	0.4841	10.3842**
RAST+skin reactivity	0.4414	0.3794	7.1129**
Log_{10} Methacholoine PC_{20} +skin reactivity	0.3517	0.2797	4.8832**
RAST+Log_{10} methacholine PC_{20} +skin reactivity	0.5386	0.4572	6.6158**

* $p<0.05$, ** $p<0.01$

Methacholine PC_{20}: Provocation concentration of methacholine to reduce FEV_1 20% below the baseline.

PD_{15}: The cummulative dose of inhaled allergen required to produce a 15% decrease in FEV_1

The Relationship Between Bronchial Reactivity and Allergic Responses

No significant relationship was found between the wormwood specific-IgE level (bound radioactivity) and methacholine PC20 of each patient (Fig. 2). As shown in Fig. 3, the threshold doses of inhaled allergen were not related to specific IgE levels presented as bound radioactivites of wormwood-RAST.Independent variables revealed that specific IgE level was the most significant factor. The multiple regression analysis on allergen PD15 was performed using three variables. The adjust R-square was highest when two variables (bound radioactivities +methacholine PC20) were taken into account, but decreased when three factors including skin reactivity were included (Table 5).

DISCUSSION

A major goal of this study was to attempt to define the characteristics of patients who develop asthma on exposure to Artemisia antigens. All patients studied had asthma and all showed significant response on the skin prick test with any one of Torri's, Bencard's or Korean Artemisia extracts. It has been proposed that the nonspecific bronchial response and specific IgE levels can be used to predict the antigenic bronchial response (Adler *et al.* 1985). Positive bronchial challenges can be found in patients if they possess sufficient IgE antibodies to ragweed(Bruce *et al.* 1975). In this study, although 7(26.9%) of those with positive challenges had wormwood-RAST class 0, patients with a high specific IgE level to Artemisia pollen had positive challenges with Artemisia antigens and patients with a positive bronchial response had significantly more IgE anti-Artemisia pollen than patients with negative challenges. IgE anti-Artemisia pollen may thus contribute to the susceptibility to Artemisia pollen challenge response.

A usual historical marker is considered to be important in the diagnosis of allergic disease and helpful in predicting challenge response. Most patients such as ragweed-sensitive patients complain of marked aggravation of their respiratory symptoms when they are exposed to ragweed pollen. In this study, only 13(50%) patients out of the patients with positive challenges noted seasonal aggravation. House dust and house dust mites are the most importnat inhalant allergens in Korea. The positive rate to house dust mites on the skin prick test (greater than 2+) was 40-60% in asthmatic patients (Yoon *et al.* 1987). There are many patients who showed positive results on the house dust bronchoprovocation test. In this study, 17(65.4%) of 26 patients with positive challenges, especially 10(76.9%) of the 13 nonseasonal type, and 5(100%) of the 5 patients with negative challenges showed concurrent positive results on the house dust bronchoprovocation test. Hong and Park (1988) already noted that the concurrent positive rate on the house dust bronchoprovocation test was very high in asthmatic patients induced by Korean pollen extracts. Also, there were 6 cases who had positive challenges with Artemisia antigen but negative challenges with house dust. These results suggest that Artemisia may be considered as one of the important

allergenic etiologies of both seasonal and perennial asthma, in which it may be an aggravating factor during the pollen season in this country.

Studies with other allergens, particularly with ragweed, have revealed evidence that nonspecific bronchial hyperreactivity as detected by methacholine or histamine inhalation is another significant determinant of response to antigenic bronchial challenge (Bryant *et al.* 1975; Nathan *et al.* 1979; Neijens *et al.* 1979; Boulet *et al.* 1983). Cockcroft *et al.* (1979) demonstrated that bronchial responsiveness to the allergen correlated positively with either cutaneous sensitivity to the allergen or bronchial responsiveness to histamine, but correlated best when both factors were taken into account. This supported the previous observation of Bryant and Burns(1976). By contrast, Popa and Garfield (1979) found a better correlation between the bronchial threshold dose for ragweed extract and for histamine than that between the bronchial threshold dose for ragweed extracts and the concentration of circulating ragweed specific IgE antibodies. In the present study, the correlation between bronchial responsiveness to methacholine and specific IgE levels was poor. Individual regressions showed that bronchial responsiveness to allergen (PD15) was more dependent upon circulating specific IgE levels to Artemisia pollen, and correlated best when two factors, specific IgE levels and bronchial responsiveness to methacholine, were taken into account. The adjusted R square decreased when three factors including skin reactivity and bronchial responsiveness to methacholine were taken into account in Table 5. As noted, nonspecific bronchial responses increase together with IgE antibody titers during a pollen season, and nonspecific bronchial responses decrease with allergen avoidance (Platts-Mills *et al.* 1982; Boulet *et al.* 1983). The two variables, however, are not independent. The nonspecific bronchial responsiveness and IgE antibodies can be used to predict the antigenic bronchial response proposed by Adler *et al.* (1985). Patients with larger amounts of specific IgE will show more skin responsiveness (Park et al. 1988). Therefore, it is possible to predict the concentration of allergen extracts required to trigger asthma by measuring two factors, methacholine PC20 and specific IgE level.

Five cases of an isolated late asthmatic response were observed in this study. The frequency of these responses has varied with different antigens and different laboratories. An association with specific IgG_4 antibodies to house dust mite has been reported (Gwynn *et al.* 1982). Thus, isolated late asthmatic reactions would be an appropriate subject for further studies of Artemisia-induced asthma.

In conclusion, IgE anti-Artemisia thus may contribute to the susceptibility to Artemisia challenges and Artemisia pollen may be considered as one of the important allergenic etiologies of atopic asthma in this country.

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