

A preceding airway reaction to one allergen may lead to priming of the airway responses to another allergen

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This study aimed to determine whether a preceding airway response to one allergen leads to priming of the airway responses to another allergen. Twelve asthmatic children who had positive prick tests to two allergens, *Dermatophagoides pteronyssinus* (D.p.) and German cockroach (CR), participated in a randomized, placebo-controlled crossover study. We performed two consecutive inhalation challenges, D.p. challenge being followed 48 h later by CR challenge. The effect of initial (D.p.) challenge on the early and late airway responses to the subsequent (CR) challenge (CR2) was examined by comparing the responses with those to CR challenge preceded by sham challenge (CR1). The geometric mean PD₂₀ of CR allergen in the CR2 was 2.8 BU (breath unit) (range of 1 SD; 0.77–10.4), which was 12.0-fold less than that (33.7 BU, 10.8–105.2) in the CR1. The administration of a 6.1-fold less dose (8.9 BU, 2.7–28.8) in the CR2 than in the CR1 (54.5 BU, 44.1–69.3) provoked a similar degree of late-phase reactions ($18.7 \pm 7.3\%$ vs $15.8 \pm 9.6\%$). Our data indicated that the early- and late-phase reactions to CR challenge were augmented by the preceding reaction to D.p. This suggests that a preceding airway response to one allergen may lead to priming, with enhancement of the early and late airway responses to the subsequent challenge with another allergen.

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Key words: *Dermatophagoides pteronyssinus*; German cockroach; heterologous allergen; primed airway responses; repeated allergen challenge.

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Airway hyperresponsiveness is characteristic of patients with asthma, and its underlying mechanism is presumed to be airway inflammation (1). This property of an increase in airway responsiveness (AR) can serve not only as an aid to diagnosis of asthma, but also as a means to assess its severity and prognosis (2). Although many subjects with asthma have a relatively stable degree of "baseline" airway hyperresponsiveness (3), AR may be further enhanced by antigenic exposure in atopic

asthma. Thus, in sensitized subjects with asthma, laboratory or natural exposure to allergens leads to an increase in nonspecific bronchial responsiveness (NSBR) as measured by responsiveness to methacholine or histamine (4, 5). Increased AR induced by allergen is associated with increased symptoms of asthma and increased need for treatment (6). These observations led to the hypothesis that a vicious cycle is established when an allergen induces airway hyperresponsiveness, which, in turn, causes an increased response to subsequent exposure to the allergen or bronchospastic triggering factors such as exercise, cold air, smoke, and dust (7).

Generally, perennial allergic asthmatics are continuously exposed to relevant allergens. The significance of repeated exposure to an allergen has been established by several investigators (8–10). For example, Ihre et al. (9) reported that repeated small doses of allergen may provoke early asthmatic

Abbreviations: AR: airway responsiveness; NSBR: nonspecific bronchial responsiveness; D.p.: *Dermatophagoides pteronyssinus*; CR: German cockroach; CR2: CR challenge preceded by D.p. challenge; CR1: CR challenge preceded by sham challenge; EAR: early asthmatic response; PC₂₀: provocation concentration which caused 20% fall in FEV₁; LAR: late asthmatic response; BU: breath unit; PD₂₀: provocation dose of allergen which caused 20% fall in FEV₁.

response (EAR) and/or late asthmatic response (LAR) in asthmatic subjects who initially showed no airway reactions. Recently, we (11) have shown that two repeated exposures to an allergen dose that was insufficient to cause significant airway reactions after a single exposure could provoke asthmatic airway responses in atopic subjects. However, it is not clear whether hyperresponsiveness of the airways to repeated allergen exposure is limited to the homologous allergen or whether the phenomenon extends to heterologous allergen exposure as well. There are currently no data published on this subject. It is a clinically relevant question because of the multiple sensitivities and concomitant exposure which are often seen in atopic asthmatics.

It is tempting to speculate that exposure of airways to one allergen leads to hyperresponsiveness at subsequent exposure to another allergen, because the response to an inhaled allergen is determined in part by the level of NSBR (12, 13), which is heightened by prior exposure to the first allergen. In an attempt to investigate and clarify the issue, we recruited atopic children with perennial asthma who were sensitive to two allergens, *Dermatophagoides pteronyssinus* (D.p.) and German cockroach (CR). In a randomized, placebo-controlled, crossover fashion, we performed two consecutive bouts of allergen challenge test, the challenge with D.p. being followed 48 h later by CR challenge, and examined the effect of initial allergen (D.p.) challenge on the early and late airway responses to the subsequent allergen (CR) challenge (CR2) by comparing the responses with those to CR challenge preceded by sham challenge (CR1).

Material and methods

Patients

Twelve children with asthma (eight boys and four girls), aged 7–13 years (mean $\pm$ SD, 10.5 ± 2.2 years), completed the main study (see Study design). Initially, 17 children with asthma took part in the study, but five were withdrawn: two subjects had such severe late-phase reactions to D.p. as to need medications, two subjects failed to show asthmatic responses to D.p., and one subject could not undergo the CR2 because of delayed recovery of airway caliber ($<90\%$ baseline, at 48 h) after the D.p. challenge test. All children had perennial asthma and positive prick test results to D.p., *D. farinae*, and CR among 20 common airborne allergens. At the time of the study, all patients had been free of acute respiratory tract infections for 4 weeks. Asthma was controlled by a β_2 -agonist on an as-needed basis, in addition to a theophylline preparation in two subjects and to inhaled beclom-

ethasone in four subjects. For assessment of possible temporal variation in airway responses to CR, a separate group of nine patients (control study group) who had the same allergenic sensitivity as those involved in the main study were recruited. Throughout the study period, all patients had to submit to concomitant medication rules; they had to stop using inhaled bronchodilators or other medications 24 h, oral theophylline 48 h, and inhaled steroid 7 days, respectively, before the study periods. The parents of the patients gave informed consent for the study, and the protocol was approved by the hospital ethics committee.

Study design (Fig. 1)

In the main study group, a randomized, crossover, placebo-controlled study was employed to investigate the effect of D.p. challenge on the early and late airway responses to inhaled CR allergen. Because of the potential risks and self-evidence of administering increasing doses of inhaled allergen (D.p.) until EARs, the study was conducted in an open fashion. Subjects attended the laboratory on 4 days, divided into two study periods, which were separated by at least 4 weeks. Each subject arrived at the laboratory at 8 a.m. On each day of the study, lung function was measured with a computerized spirometer (Microspiro-HI 298, Chest, Japan) after a rest of 30 min, and the study was continued only if the baseline FEV₁ before each test was at least 70% of the predicted value (14). The largest value of the triplicate FEV₁ at each time was used for analysis. On visit 1, subjects underwent a methacholine challenge test. Two hours later, once the airway caliber had recovered (more than 90% of the baseline), they were challenged with either D.p. or placebo inhalation, and FEV₁ was measured at hourly intervals for 10 h. During visit 2, which took place 2 days later, methacholine responsiveness was remeasured, and the allergen challenge test with CR was undertaken. FEV₁ was measured as in visit 1. In the second period, the same protocol as in the first period was repeated except that the first challenges in visit 3 were crossed-over from those of visit 1. Methacholine or allergen bronchoprovocation tests were performed such that each specific agent was given at the same time of the day. In the control study group, two CR challenges were performed so that the first and the second challenges fell on the first and the second study periods, respectively, of the main study group.

Methacholine inhalation test

Methacholine bronchial challenges were carried out by a modification of the method described by

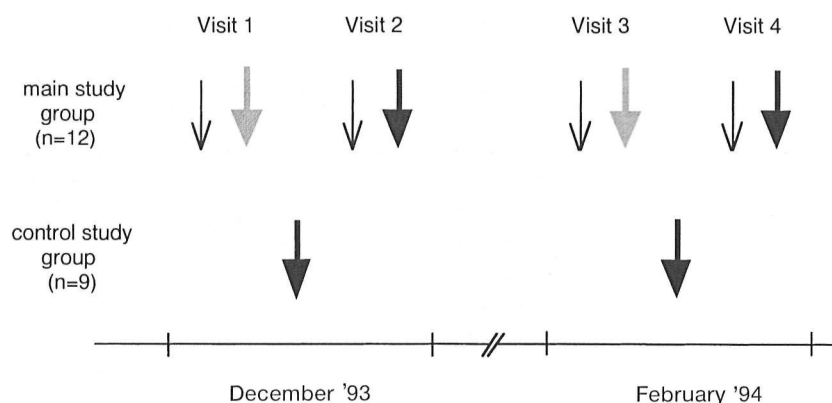


Fig. 1. Schematic flow chart of study design. Interval between each visit in one period was 2 days. Interval between two periods was at least 4 weeks. ↓Methacholine challenge test performed 2 h before each allergen challenge test. ↓*Dermatophagoides pteronyssinus* or sham challenge test. ↓German cockroach challenge test.

Chai et al. (15). The concentrations (0.075, 0.15, 0.3, 0.625, 1.25, 2.5, 5, 10, and 25 mg/ml) of methacholine (Sigma Chemical, St Louis, MO, USA) were prepared with dilution in buffered saline (pH 7.4). A Rosenthal-French (Laboratory for Applied Immunology, Baltimore, MD, USA) dosimeter, triggered by a solenoid valve set to remain open for 0.6 s, was used to deliver the aerosol generated from a DeVilbiss 646 nebulizer with pressurized air at 20 psi. Each subject inhaled five inspiratory capacity breaths of buffered saline and increasing concentrations of methacholine until FEV₁ had fallen by >20% of the postsaline value. FEV₁ was measured 3 min after each inhalation. The concentration of methacholine which caused a 20% fall in FEV₁ (PC₂₀) was obtained from the log concentration-percent fall in FEV₁ curve by linear interpolation of the last two points.

Allergen challenge test

Allergen challenge tests were performed by a simple modification of the method described by Chai et al. (15). The extracts of D.p. were obtained from Bencard, UK, and serial alternative five- and twofold dilutions were prepared with buffer phosphate (10^{-3} , 2×10^{-4} , 10^{-4} , 2×10^{-5} , and 10^{-5} w/v concentrations). The extracts of CR (*Blattella germanica*) were prepared by a modification of the method of Bernton & Brown (16), and appropriately diluted (10-fold) (2.5 mg/ml, and 250, 25, and 2.5 µg/ml) from the concentrated extract (25 mg/ml). The allergen concentrations were inhaled after a control inhalation of buffer phosphate, starting with the lowest concentration. The baseline values of each allergen test were FEV₁ measurements obtained after inhaling buffer solutions just before allergen exposure. Aerosols were generated as in the methacholine challenge. FEV₁ was measured at 10 min after the inhalation of five inspiratory

capacity breaths of each concentration. Inhalations were continued at 10-min intervals until there was a 20% fall or more from baseline FEV₁, or the maximal concentration was reached. If the fall in FEV₁ was between 15% and 20%, the next increment was half the usual concentration. For the sham challenge, the allergen diluent, buffer phosphate, was inhaled five times with inspiratory capacity breath on three occasions at 10-min intervals. After the last concentration, FEV₁ was measured at hourly intervals for 10 h. The changes in FEV₁ after allergen inhalation were expressed as percentage of baseline. Response was calculated as Δ FEV₁ (percent fall in FEV₁ from baseline), measured at 10 min after the last concentration of allergen (magnitude of the early response), and the maximal Δ FEV₁ was measured between 3 and 10 h after the allergen inhalation (magnitude of the late response). The EAR or LAR was, respectively, defined by a magnitude of the early or late response greater than 20%.

The doses of CR allergen administered were expressed as breath units (BUs), 1 BU corresponding to one breath of 250 µg/ml of CR allergen. The provocation dose of CR allergen which caused a 20% fall in FEV₁ (PD₂₀) was then calculated by linear interpolation of the last two points on the log cumulative dose-percent fall in FEV₁ curve, or by extrapolation of the last two data points when FEV₁ did not fall by more than 20% despite administration of the highest concentration.

In the control study group, the series of CR allergen concentrations was determined by the first challenge; for each specific subject, the same dose of allergen was administered in the second challenge.

IgE ELISA inhibition test

In order to examine the cross-reactivity between D.p. and CR, the IgE ELISA inhibition test was

done by a modification of the method of Lehrer et al. (17). An amount of 2 µg of D.p. extract used in the inhalation challenge test was coated on the ELISA plate. For the ELISA inhibition test, 100 µl of variable dilutions of inhibitor, D.p. or CR, was incubated with 50 µl of undiluted individual sera of three patients sensitive only to D.p., for 4 h at room temperature. After washing the antigen-coated wells three times with phosphate-buffered saline (pH 7.4) containing 0.05% Tween 20, 100 µl of each solution was incubated for 2 h at 37°C. For the remaining steps of the assay, biotinylated anti-human IgE (1 : 1000 diluted, Vector, USA), horseradish peroxidase streptavidin (1 : 1000 diluted, Vector, USA), and substrate containing 2,2-azino-bis-3-methylbenzothiazoline-6-sulfonic acid (Vector, USA) were used. The optical density was read at 410 nm with an ELISA reader (Dynatech MR300), and to detect cross-allergenicity, we estimated ID₅₀ (inhibitory dose that would inhibit 50% of the ELISA) graphically by plotting the different dilutions of test material.

Analysis

The changes in methacholine responsiveness after D.p. or sham challenge were expressed as $\Delta \log PC_{20} [\log_2 (PC_{20} \text{ 2 days after challenge}) - \log_2 (PC_{20} \text{ baseline})]$, and any change in PC₂₀ of methacholine greater than twofold ($\Delta \log PC_{20} > 1$ or < -1) was regarded as significant. Mean values for PC₂₀ of methacholine and PD₂₀ or administered cumulative dose of CR were expressed as geometric means (range of 1 SD). Other values were presented as mean $\pm$ 1 SD. Comparisons of the values between the CR1 and CR2 were made by the Wilcoxon signed-ranks test. In each case, statistical significance was accepted at $P < 0.05$. In the control study group, reproducibility of the magnitude of early- and late-phase reaction after CR challenge was examined by calculating intraclass correlation coefficients.

Results

The bronchial responses to a sham or D.p. challenge and the subsequent CR challenges in the 12 subjects are shown in Table 1. Seven subjects and five subjects underwent the sham challenge and the D.p. challenge, respectively, in the first period. In the D.p. challenge, one subject (no. 7) needed up to half the fourth concentration, two subjects (nos. 5 and 9) up to the fourth concentration (2×10^{-4} w/v), one subject (no. 11) up to half the final concentration, and the other eight subjects up to the final concentration (10^{-3} w/v) to have EARs. In the CR challenge preceded by sham challenge (CR1), two

subjects (nos. 5 and 7) needed up to half the final concentration to have EARs, and one subject (no. 1) who had no EAR with the above concentration needed more than the final concentration (2.5 mg/ml). The other nine subjects reached the final concentration by the usual dose step. In the CR challenge preceded by D.p. challenge (CR2), four subjects (nos. 2, 5, 7, and 11) needed up to half the third concentration, four subjects (nos. 1, 8, 10, and 12) up to the third concentration (250 µg/ml), two subjects (nos. 4 and 6) up to half the final concentration, and the other two subjects up to the final concentration (2.5 mg/ml) to have EARs.

There was no significant change in FEV₁ after the sham challenge. Comparison of methacholine PC₂₀ between before and 2 days after this challenge showed no significant change in any subject. In the CR1, at the early phase and late-phase, FEV₁ decreased to $74.7 \pm 8.3\%$ and $84.2 \pm 9.6\%$, respectively, of the baseline value. With the dose of 54.5 BU (geometric mean; range of 1 SD, 44.1–69.3 BU) administered, 10 subjects showed EAR, but only three subjects showed LAR. The geometric mean of PD₂₀-CR was 33.7 BU (10.8–105.2 BU). In the D.p. challenge, all the subjects showed EAR, and nine subjects showed LAR as well. Two days after D.p. challenge, methacholine PC₂₀ decreased by 1.28 ± 0.65 in doubling dilutions. In the CR2, at the early phase and the late phase, FEV₁ decreased to $70.8 \pm 4.8\%$ and $81.3 \pm 7.3\%$ of the baseline value. With the dose of 8.9 BU (2.7–28.8 BU) administered, all the subjects showed EAR, and five subjects showed LAR as well. The geometric mean of PD₂₀-CR was 2.8 BU (0.77–10.4 BU) (Table 1).

Methacholine PC₂₀ before the CR2 (1.40 mg/ml, 0.62–3.18) was significantly ($P < 0.01$) lower than that (3.74 mg/ml, 1.93–7.24) before the CR1. Comparison of the two CR challenges showed that baseline FEV₁ ($86.8 \pm 6.4\%$ predicted) in the CR2 was significantly ($P < 0.01$) lower than that ($93.1 \pm 8.0\%$ predicted) in the CR1. The early-phase or late-phase reaction was comparable between the two CR challenges. PD₂₀ and the administered dose of CR in the CR2 were significantly ($P < 0.01$) lower than the corresponding values in the CR1 (Table 1).

Two subjects who showed only EAR to CR1 were converted into dual responders in the CR2 (Fig. 2). These subjects had shown LAR to preceding D.p. challenge.

The data of CR challenges in the control study group are shown in Table 2. The magnitude of the early-phase response was reproducible (intraclass correlation coefficient: 0.80), as was the magnitude of the late-phase response (intraclass correlation coefficient: 0.83).

In the IgE ELISA inhibition test (Fig. 3), the D.p. ELISA was significantly inhibited by the D.p.

Table 1. Allergen bronchoprovocation data of main study group

Subject no.	Sham challenge test				CR challenge test (CR1)					
	PC ₂₀ * (mg/ml)	Baseline FEV ₁ †	Early phase FEV ₁ ‡	Late phase FEV ₁ ‡	PC ₂₀ * (mg/ml)	Baseline FEV ₁ †	Early phase FEV ₁ ‡	Late phase FEV ₁ ‡	PD ₂₀ § (BU¶)	Administered dose (BU¶)
1	6.2	107.2	97.4	102.6	4.9	107.2	56.1	66.5	32.6	80.6
2	2.8	96.4	102.5	97.5	4.4	93.7	77.9	88.3	37.5	55.6
3	7.5	99.5	98.5	98.2	9.6	97.7	87.1	96.4	334.5	55.6
4	4.6	86.1	96.3	94.7	2.8	83.4	86.7	92.4	260.4	55.6
5	1.8	95.3	97.8	95.6	2.6	95.3	71.8	86.8	9.8	30.6
6	6.8	103.8	97.6	95.2	3.3	106.3	77.0	88.5	35.0	55.6
7	0.76	92.2	101.9	98.1	0.92	94.9	68.3	73.9	8.0	30.6
8	6.9	89.6	98.7	97.3	9.6	87.3	73.6	93.5	23.8	55.6
9	1.9	87.4	103.1	101.5	2.8	89.1	73.0	87.9	17.6	55.6
10	4.7	80.7	95.8	97.9	6.4	82.8	77.5	69.2	33.6	55.6
11	3.2	90.6	96.7	95.0	2.1	86.2	69.4	84.7	15.3	55.6
12	3.5	89.9	100.0	97.8	4.5	93.7	78.5	82.3	35.0	55.6
Mean	3.53#	93.2	98.8	97.6	3.74#	93.1	74.7	84.2	33.7#	54.5#
SD	(1.77–7.00)	7.6	2.5	2.4	(1.93–7.24)	8.0	8.3	9.6	(10.8–105.2)	(44.1–69.3)
	D.p. challenge test				CR challenge test (CR2)					
	PC ₂₀ * (mg/ml)	Baseline FEV ₁ †	Early phase FEV ₁ ‡	Late phase FEV ₁ ‡	PC ₂₀ * (mg/ml)	Baseline FEV ₁ †	Early phase FEV ₁ ‡	Late phase FEV ₁ ‡	PD ₂₀ § (BU¶)	Administered dose (BU¶)
1	7.8	103.7	76.1	86.7	2.6	96.8	78.5	85.7	4.1	5.6
2	2.6	92.4	66.3	74.1	1.2	85.7	71.0	68.3	0.95	3.1
3	4.5	94.9	79.8	94.5	3.8	91.5	73.7	88.8	27.2	55.6
4	3.3	83.4	67.4	79.6	2.2	79.6	75.0	86.1	11.2	30.6
5	1.5	93.2	66.6	79.9	0.93	87.0	67.9	90.8	0.87	3.1
6	5.2	103.2	69.1	66.8	1.4	100.2	63.8	80.7	7.8	30.6
7	0.84	92.5	74.8	53.4	0.32	84.0	65.7	73.7	0.55	3.1
8	12.5	87.3	76.2	82.8	6.8	82.2	77.9	87.6	3.4	5.6
9	1.5	87.0	67.7	66.1	0.81	80.4	69.6	82.4	12.5	55.6
10	4.1	89.2	70.3	66.3	1.5	86.6	71.7	71.7	1.4	5.6
11	1.7	88.4	74.9	59.9	0.69	81.5	64.8	75.3	0.69	3.1
12	6.3	90.4	71.4	69.2	0.92	86.3	70.1	83.9	1.4	5.6
Mean	3.30#	92.1	71.7	73.3	1.40#	86.8	70.8	81.3	2.8#	8.9#
SD	(1.49–7.26)	6.2	4.5	11.8	(0.62–3.18)	6.4	4.8	7.3	(0.77–10.4)	(2.7–28.8)

* Provocation concentration of methacholine which caused 20% fall in FEV₁ from baseline. It was measured immediately before each allergen challenge test.

† % predicted for height (14).

‡ % baseline.

§ Provocation dose of CR allergen which caused 20% fall in FEV₁ from baseline. It was calculated as cumulative breath unit.

¶ Breath unit equal to one inhalation of 250 µg/ml CR allergen.

Geometric mean (range of 1 SD).

extract (ID₅₀ was 5.9, 6.8, and 4.7 µg/ml, respectively), but not by the CR extract.

Discussion

In this randomized, placebo-controlled, crossover study, we set out to examine the effects of one preceding allergen exposure on the airway responses to another allergen. The geometric mean PD₂₀ of CR allergen in the challenge preceded by an airway reaction to D.p. was reduced by 12.0-fold (2.8 BU/33.7 BU), compared to that without preceding allergen exposure. The use of a constant-dose allergen challenge protocol, as opposed to the traditional method of measuring the PD₂₀ to allergen such as ours, is necessary when a measurement of the effects of treatment on the late

response is desired. However, the administration of a reduced dose of CR allergen by 6.1-fold (8.9 BU/54.5 BU) in the former challenge provoked a degree of late-phase reaction similar to that in the latter challenge. Furthermore, two subjects who showed EAR only to the CR1 were shown to be converted into dual responders in the CR2 despite a substantially reduced inhaled allergen dose. These data suggest that late-phase reaction to CR challenge was also augmented by the preceding reaction to D.p. because the occurrence and magnitude of LAR are dependent on allergen dose (18).

Our data indicate that the early and late reactions to CR challenge were augmented by the preceding reaction to D.p. There have been several reports (8–11) suggesting that repeated exposure

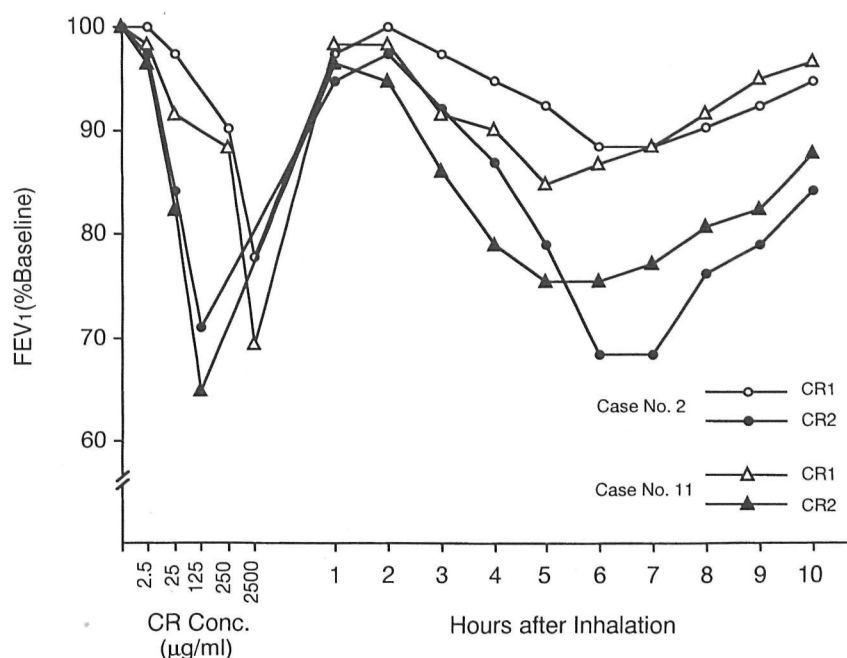


Fig. 2. Change of FEV₁ after German cockroach (CR) extract inhalation in two patients (nos. 2 and 11). Each patient displayed only EAR in sham period, but was converted into dual responder in D.p. period.

Table 2. Data of two CR (German cockroach) challenge tests in control study group

Subject no.	CR challenge test (I)			CR challenge test (II)		
	Baseline FEV ₁ †	Early phase FEV ₁ ‡	Late phase FEV ₁ ‡	Baseline FEV ₁ †	Early phase FEV ₁ ‡	Late phase FEV ₁ ‡
1	81.9	78.7	71.2	85.3	76.3	75.5
2	83.9	77.6	81.6	85.6	71.0	74.0
3	108.8	57.4	55.3	105.7	64.9	62.1
4	106.3	67.6	64.7	109.5	60.0	74.9
5	87.5	73.2	70.7	89.6	76.2	78.6
6	89.4	71.4	64.3	89.4	64.9	54.4
7	96.7	83.9	88.5	96.7	89.4	86.3
8	91.6	81.1	93.8	88.2	80.6	89.2
9	86.0	74.3	90.4	83.1	72.1	84.8
Mean	92.5	73.9	75.6	92.6	72.8	75.5
SD	9.6	8.0	13.5	9.3	9.0	11.3

† % predicted for height (14); ‡% baseline.

to the same allergen primes the airway responses. Our results suggest that prior exposure to one allergen may lead to priming with enhancement of the early and late responses on subsequent exposure to another allergen. Although it is apparent that exposure to one allergen may increase the responsiveness to another allergen in subjects who are allergic to both, it has not been proven by a proper study in the past. To our knowledge, this is the first study to show that the occurrence of priming to repeated allergen exposure extends to the heterologous allergen as well under experimental conditions; that is, a significant reaction to

one relevant allergen may be followed by exaggerated responses to another allergen.

The increased airway responses to the CR2 may have been due to a reduced airway caliber. Although airway caliber could theoretically influence measurements of airway responses to allergen (19), the strength of this effect is a matter of controversy, and some investigators (20, 21) have reported that even moderate changes in airway caliber do not affect the measurement of NSBR. Although statistically significant, the mean difference in baseline FEV₁ between the CR1 and CR2 was only 6.3% of the predicted. Therefore, we

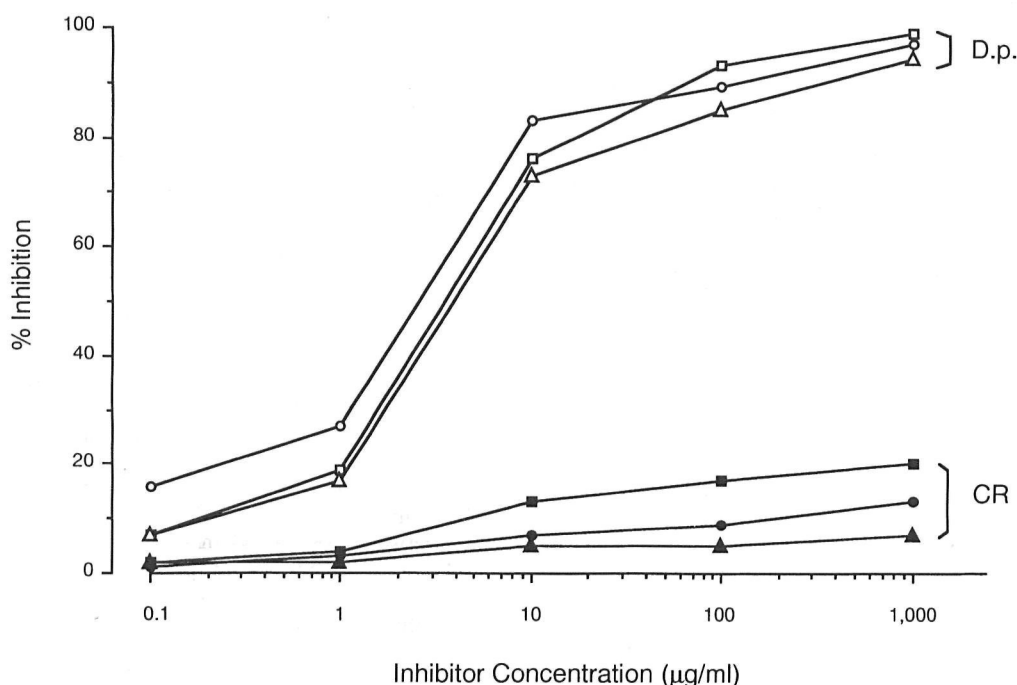


Fig. 3. Inhibition of *Dermatophagoides pteronyssinus* (D.p.) ELISA in three D.p.-sensitive subjects. Increasing concentrations of D.p. and German cockroach (CR) extracts were used to inhibit D.p. ELISA.

believe that such a baseline effect would not account entirely for the increased airway responses to the CR2.

The phenomenon may have been due to cross-reactivity (22) between the two allergens used. This is unlikely, however, because our *in vitro* data showed no allergenic cross-reaction between D.p. and CR. These allergens were selected for our study, because they are common inhalant allergens in Korea (23). We admit that the degree of continual exposure to these allergens in the environment may have changed during the study period. This potential variability, however, would not change the figure, because this study was performed between December and February, when the levels of these allergens have been found to be the lowest and least changing (24, 25). Furthermore, duplicate CR challenges in the control study group performed in the coincident period showed good reproducibility and no significant temporal variation. We used D.p. for the first challenge, because this allergen induces more airway reaction than CR and to that extent may make clear the difference of airway reaction to the subsequent CR challenge.

The mechanism by which the priming by one allergen to another allergen occurs is not clear. Forty-eight hours was chosen as the interval for the two consecutive allergen challenges because it represents an appropriate time point to allow airway caliber to return to baseline and minimize the diurnal variation of airway reactions to the allergen

challenge (26, 27). At this time, the PC_{20} of methacholine before the CR2 was 2.67-fold lower than that before the CR1. This may partly explain the increased early-phase reaction and late-phase reaction to the CR2, because both are related to NSBR (28, 29). However, the marked decrease of PD_{20} (12.0-fold) or administered dose of allergen (CR) to elicit the same degree of late-phase reaction (6.1-fold) compared with the magnitude of the fall in methacholine PC_{20} suggests that mechanisms other than NSBR may also operate in the increase of AR to allergen. We speculate that an immunologic priming effect on the heterologous allergen is involved. For example, various cytokines or inflammatory mediators formulated by the initial allergen challenge may enhance the functional responsiveness of mast cells to stimulation with specific IgE and antigen (30). The number of target cells, including residual mast cells (31) and basophils (32), available for the second challenge may also be increased. In this study, we think it is very difficult to distinguish the immunologic priming effect from the increased NSBR as the mechanism of heightened airway responses to the second allergen. One way to distinguish between them would be to perform the second challenge after complete recovery from NSBR.

The possibility that the current results may be of clinical relevance remains intriguing. It is unlikely that airways are exposed to a dose of allergen as high as the dose used for these allergen challenges.

Therefore, these challenges do not precisely mimic natural exposure. It would be clinically more relevant to test whether an increase in clinical sensitivity to one allergen occurs after prior exposure to low doses of another allergen. The study protocol (33), however, is more difficult to perform than traditional allergen challenges used in the present study. Nonetheless, the phenomenon observed in this study may have provided some insights into the mechanisms involved in the development of asthmatic symptoms in atopic asthma with multiple sensitivities. For example, our findings may account for the clinical observation that asthmatic patients allergic to both pollen and a second allergen are more prone to asthmatic symptoms on contact with the second allergen during the pollen season than during the rest of the year (34). Our results suggest that patients may respond to an allergen not otherwise considered clinically significant if they had been exposed to or primed by a clinically significant allergen. We speculate that multiply sensitive asthmatics may benefit clinically if some measures to avoid or remove one allergen are instituted in circumstances where it is impossible to remove all relevant allergens.

In conclusion, our data indicate that the early- and late-phase reactions to CR challenge were augmented by the preceding reaction to D.p. This suggests that a preceding airway response to one allergen may lead to priming with enhancement of the early and late airway responses to subsequent challenge with another allergen. Our results also suggest that multiple relevant allergens to which asthmatic patients are exposed in the environment may act in concert with each other on asthmatic symptoms.

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