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Bronchial allergen challenge in subjects with low levels of allergic sensitization to indoor allergens

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Date:

Accepted for publication 5 November 1998

To cite this article:

Witteman A.M., Mulder M., Aalberse R.C., Jansen H.M.,
van der Zee J.S. Bronchial allergen challenge in subjects
with low levels of allergic sensitization to indoor
allergens.
Allergy 1999; 54, 366–374.

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ISSN 0105-4538

Key words: bronchial allergen challenge; RAST; skin test.

Background: Low skin reactivity to common inhalant allergens is frequently found in asymptomatic individuals as well as in patients with respiratory complaints. However, most studies on bronchial allergen challenge concern patients with high levels of allergic sensitization. The present study was directed to bronchial reactions after allergen challenge in subjects with low skin reactivity to *Dermatophagoides pteronyssinus* or cat dander.

Methods: Titrated intracutaneous skin tests, skin prick tests, specific IgE assays, histamine release on washed leukocytes, and bronchial histamine and allergen-challenge tests were performed in 20 subjects with an intracutaneous skin test threshold for cat dander (*Felis domesticus*) or *D. pteronyssinus* above 0.1 BU/ml (mean wheal diameter in skin prick test with 10 000 BU/ml: 4.4 mm). Ten of the 20 patients had specific IgE below the detection limit in at least one of the three IgE assays which were done. Fifteen patients had a specific IgE level below 2 kU/l in all three tests. As a positive control group, the same parameters were studied in seven moderately sensitized patients with an intracutaneous skin test threshold below 0.1 BU/ml (mean wheal diameter with 10 000 BU/ml: 7.2 mm).

Results: The 20 subjects with low levels of allergic sensitization had an early decrease in FEV₁ of 8.6% ($P < 0.01$) and a mean late decrease of 6.3% ($P < 0.05$). There was a trend for decrease in PC₂₀ histamine 24 h after allergen challenge (–0.4 doubling doses, $P = 0.09$).

Conclusions: In this group of subjects with low levels of allergic sensitization, a statistically significant early and late decrease in FEV₁ was found. However, the decrease in lung function was small and unnoticed by most patients. The increase in nonspecific bronchial hyperresponsiveness after bronchial allergen challenge did not reach statistical significance in the study

group. The results indicate that allergen exposure in patients with low levels of allergic sensitization may lead to airways changes in the absence of acute symptoms.

Skin test and RAST are mostly used to diagnose allergy. The overall agreement between the tests is 75–95% (1–3). Discrepancies between skin test and RAST are usually a positive skin test without detectable IgE antibodies in serum. The clinical relevance of these discrepancies is not clear. In an earlier study, we found most discrepancies between the two tests in patients with an intracutaneous skin test threshold above 0.1 BU/ml (3).

To learn more about the clinical relevance of such a low degree of allergic sensitization, we measured bronchial reactions after allergen challenge in a group of 20 subjects with an intracutaneous skin test threshold for *Dermatophagoides pteronyssinus* or cat dander above 0.1 BU/ml. Seven moderately sensitized patients with skin test thresholds below 0.1 BU/ml served as positive control group.

Bronchial allergen challenges were used as a model for bronchial reactions under natural allergen exposure. Allergen challenge often leads to early and late allergic reactions and induction of nonspecific bronchial hyperresponsiveness (4).

The early allergic reaction in the airways is an episode of airflow obstruction immediately after allergen exposure and reaches its maximum within 1 h. The early allergic reaction is mainly caused by IgE-dependent mast-cell degranulation (5).

Allergen-induced increase in nonspecific bronchial responsiveness is associated with the late reaction (6, 7). Bronchial responsiveness increases at 2–3 h after exposure, is greatest after 24 h, and may persist for several days (8, 9).

The late allergic reaction is an episode of airflow obstruction occurring 4–8 h after allergen exposure (10). The pathogenesis of the late allergic reaction is, as yet, not fully known. Studies in man have shown that induction of bronchial hyperresponsiveness and late allergic reactions are associated with an increase in bronchial inflammation, particularly by influx of eosinophils (11).

It has been claimed that the allergen concentration which causes a 20% fall in FEV₁ in the first hour after allergen challenge (PD₂₀ early) can be predicted from the level of allergy and the bronchial responsiveness to inhaled histamine or methacholine (12, 13). The level of allergy is determined by the skin test or by the level of specific IgE antibodies. Less is known about the prediction of the late

allergic reaction and the relation with induction of bronchial hyperresponsiveness. Important determinants appear to be high concentrations of specific IgE, magnitude of the early allergic reaction, and the initial level of bronchial responsiveness (14, 15). In this respect, the role of the total allergen dose may also be important. Some authors showed that inhalation of high doses of allergen resulted in an increase of late reactions (16), and others indicated that isolated late allergic reactions and induction of bronchial hyperresponsiveness may occur after repeated inhalation of low doses of allergen (17).

Material and methods

Patients

Low levels of sensitization to allergen were defined as intracutaneous skin test thresholds (allergen concentrations evoking erythema reaction of 10 mm) for *D. pteronyssinus* or cat dander above 0.1 BU/ml.

The group consisted of patients with asthma ($n=10$), rhinitis ($n=5$), and asymptomatic subjects ($n=5$). The group with allergic asthma and/or rhinitis were selected from consecutive new patients of the outpatient departments of pulmonology and otorhinolaryngology. Asthmatics had a history of asthma as defined by the criteria of the American Thoracic Society (18): episodes of dyspnea and/or wheezing. The subjects with rhinitis had symptoms of watery rhinorrhea, nasal blockage, sneezing attacks, or nasal pruritus without current or past symptoms of dyspnea or wheezing. The asymptomatic subjects were volunteers with positive skin tests without symptoms of the airways.

Moderate sensitization in seven patients was defined as an intracutaneous skin test threshold below 0.1 BU/ml (geometric mean 0.017 BU/ml, geometric standard deviation [GSD] 2.2). Five of them had asthma and two complaints of perennial rhinitis.

Medications for asthma were short-acting β_2 -agonists and inhaled corticosteroids. Inhalation corticosteroids were stopped 2 weeks before the bronchial challenge test. No short-acting β_2 -agonists were taken at least 8 h before the bronchial challenge test. The patients with rhinitis and the asymptomatic subjects did not use medication.

The study was approved by the AMC Medical Ethics Committee, and written informed consent was given by all patients. The subject characteristics are given in Table 1.

Table 1. Subject characteristics

	Sex	Age (years)	Symptoms	Smoking	FEV ₁ (% predicted)	PC ₂₀ histamine (mg/ml)
Subjects with skin test thresholds above 0.1 BU/ml						
1	M	27	R*	Ex	120	22.0
2	F	57	A†	Yes	65	2.2
3	F	26	AR	No	97	4.9
4	F	42	A	Ex	109	7.0
5	M	35	AR	No	73	12.8
6	F	27	–	No	93	9.5
7	F	29	R	No	119	13.1
8	M	22	AR	No	104	2.4
9	M	19	–	No	99	21.8
10	F	20	–	No	96	6.0
11	M	42	–	No	115	>32.0
12	F	30	A	No	94	5.6
13	F	32	–	Yes	115	>32.0
14	F	28	AR	Ex	123	1.0
15	M	42	A	Ex	98	1.4
16	M	42	A	Ex	115	6.6
17	M	33	R	No	85	22.6
18	F	29	R	No	118	>32.0
19	F	23	R	No	89	>32.0
20	F	27	AR	Ex	82	0.9
Mean (SEM)		32			100.5 (3.7)	
Geometric mean						7.9
*R=rhinitis; †A=asthma.						

RAST and total IgE

Three different RAST assays were performed. In the *in vivo* reagents RAST (IVR-RAST), allergens from the same production batch were used as in the intracutaneous skin test and bronchial challenge test (ALK-Benelux, Groningen, The Netherlands). After dialysis, *D. pteronyssinus* and cat dander were insolubilized with CNBr-activated Sepharose (2 mg/100 mg Sepharose). The RAST was performed as described earlier (19). For routine detection of IgE antibodies (routine RAST), we used the same procedure, but allergen extracts were obtained from another manufacturer (HAL, Haarlem, The Netherlands). A standard curve was made by measuring binding of a reference serum in a two-site assay with Sepharose-coupled sheep-antihuman IgE and ¹²⁵I-labeled anti-IgE. The detection limit was 0.1 kU/l.

In addition, Pharmacia-RAST (CAP) (Pharmacia, Uppsala, Sweden) was performed according to the manufacturer's instructions. The sensitivity of the assay is 0.35 kU/l. All

RAST tests were performed in duplicate. Total serum IgE was quantified by an inhibition assay, as described earlier (20).

Histamine release

The isolation of human leukocytes and incubation of the leukocytes with allergen (highest concentration 1000 BU/ml) and anti-IgE (20 ng/ml) were performed by the method of Lichtenstein & Osler (21) with slight modifications. The anti-IgE was a polyclonal sheep anti-serum (CLB no. SH25P01). The erythrocytes were lysed with isotonic ammonium chloride at 4°C (22). The cells were separated by centrifugation and washed in medium containing 132 mM NaCl, 6 mM KCl, 1 mM CaCl₂, 1 mM MgSO₄, 1.2 mM potassium phosphate, 20 mM HEPES (Sigma Chemicals, St Louis, MO, USA), 5.5 mM glucose, and 0.5 % (w/v) human albumin, pH 7.4. A 250-μl cell suspension was incubated with 50 μl of the stimulus at 37°C for 1 h.

Table 2. Skin test results

		Allergen	i.c. skin threshold	i.c. (30 BU/ml)		Prick test (10 000 BU/ml)		Prick test histamine (10 mg/ml)	
				Wheal (mm)	Flare (mm)	Wheal (mm)	Flare (mm)	Wheal (mm)	Flare (mm)
			(BU/ml)						
1	Mite	13		9	27	3	11	5	28
2	Mite	13		9	27	3	11	6	37
3	Mite	11		9	33	9	24	9	37
4	Mite	1.8		9	34	2	14	6	28
5	Mite	1.6		15	30	5	10	7	11
6	Mite	1.2		11	41	6	26	6	28
7	Mite	1.2		10	33	4	18	5	18
8	Mite	1.1		11	31	7	28	5	22
9	Mite	1.1		9	42	2	16	4	21
10	Mite	0.96		na	na	5	28	6	28
11	Mite	0.13		12	37	9	27	8	37
12	Mite	0.11		6	18	3	11	8	30
13	Mite	0.11		12	38	7	30	9	23
14	Cat	12		9	29	3	11	11	35
15	Cat	3.0		8	15	2	4	6	26
16	Cat	1.0		16	42	4	10	7	30
17	Cat	0.94		14	47	na	na	9	35
18	Cat	0.17		11	39	3	10	6	20
19	Cat	0.14		na	na	8	32	5	20
20	Cat	0.13		13	35	4	28	9	22
Mean				10.7	33.2	4.4	17.4	6.8	26.8
Geometric mean			1.0						
i.c.: intracutaneous; na: not available.									

Histamine in the supernatant of the samples was measured with an automated fluorometric method (23). The results were expressed as the percentage of the totally released histamine, minus the spontaneous release. By interpolation of the log-linear dose-response curve, the allergen concentration causing 10% release was calculated. Patients with a histamine release of less than 10% for allergens and anti-IgE were considered nonresponders.

Skin tests

Skin prick tests and intracutaneous tests with cat dander and house-dust mite (ALK-Benelux, Groningen, The Netherlands) were done in the forearm. For the intracutaneous test, an end-point titration was performed by injecting 0.02 ml of 10-fold dilutions, starting with 30 BU/ml. The reactions were read after 15 min. The wheal and erythema sizes were calculated as the mean of the

largest wheal diameter and that perpendicular to it. A threshold concentration eliciting an erythema reaction of 10 mm was calculated by log-linear interpolation. An earlier study showed a high correlation ($r=0.98$, $P=0.001$, $n=59$) between the thresholds measured by wheal (5 mm) and flare (10 mm) (24). The control test with dilution buffer was negative in all. Histamine sensitivity was expressed as mean diameter of erythema and wheal in the prick test with 10 mg/ml histamine diphosphate. The skin prick test with allergen was done with 10 000 BU/ml.

Bronchial challenge tests

A histamine-challenge test was performed by the method of Cockcroft et al. (25). Histamine aerosol was inhaled by quiet tidal breathing by means of a De Vilbiss nebulizer (output 0.13 ml/min) for 2 min. The first aerosol inhaled was the control diluent (phosphate-buffered saline), and it was

Table 3. *In vitro* allergy test results

	Allergen	RAST (IVR) kU/l	RAST (routine) kU/l	RAST (Pharmacia) kU/l	Total IgE kU/l	Histamine-release threshold BU/ml
1	Mite	neg	neg	neg	58	30.7
2	Mite	neg	1.1	0.5	173	nr*
3	Mite	0.2	0.3	1.0	99	nr
4	Mite	neg	neg	neg	14	50.0
5	Mite	0.9	neg	0.6	101	nr
6	Mite	0.3	1.0	0.8	3	0.6
7	Mite	1.3	1.0	1.3	466	500.0
8	Mite	0.9	neg	0.6	298	293.2
9	Mite	1.1	1.7	2.2	139	307.1
10	Mite	2.4	2.4	4.1	63	4.6
11	Mite	3.0	2.3	5.6	150	7.0
12	Mite	neg	0.3	neg	14	nr
13	Mite	0.7	0.2	0.9	55	7.8
14	Cat	0.6	neg	neg	803	354.3
15	Cat	neg	neg	neg	94	nr
16	Cat	10.0	0.3	1.4	1000	5.9
17	Cat	neg	neg	neg	128	na†
18	Cat	17.8	neg	3.6	233	0.8
19	Cat	0.6	0.7	0.7	694	nr
20	Cat	0.3	0.3	0.5	396	517.6
Mean		2.0‡	0.6	1.2		
Geometric mean					119.7	28.7
neg: negative; *nr: nonreleaser; †na: not available. ‡Negative results were allotted half of detection limit (0.05 kU/l) for statistical analysis.						

followed by doubling concentrations of histamine from 0.015 to 32 mg/ml at 5 min. The FEV₁ was measured with a wet spirometer (Sensor Medics BV, Biltoven, The Netherlands) before the test and 30 and 90 s after each inhalation step. The test was stopped if the FEV₁ had fallen 20% or more from the baseline value. The results were expressed as the concentration of histamine causing a 20% fall in FEV₁ (PC₂₀). The PC₂₀ was calculated by interpolation of the logarithmic dose-response curve. The log ratio PC₂₀ 48 h before and 24 h after allergen challenge was used as measure of the induction of bronchial hyperresponsiveness. In addition, terminal slopes of the dose-response curve were compared in order to circumvent censored data in patients with a threshold above 32 mg/ml.

The test procedure was completed in 4 consecutive days. On day 1, after blood sampling, skin tests were performed and spirometry with histamine threshold was measured; on day 2, the challenge procedure with control solution for establishing diurnal variation was done; on day 3, the

allergen challenge was done; on the morning of day 4, spirometry and histamine threshold were repeated.

Allergen was inhaled by tidal breathing (1 min at 15-min intervals) with fivefold stepwise increasing concentrations (80–10 000 BU/ml) of cat dander or *D. pteronyssinus* extract (ALK-Benelux, Groningen, The Netherlands), with a De Vilbiss Nebulizer (output 0.13 ml/min). The extracts were diluted in phosphate-buffered saline with 0.03% human serum albumin and 0.5% phenol (ALK-Benelux, Groningen, The Netherlands). If a drop of more than 15% from the initial value of FEV₁ occurred, no additional allergen inhalations were administered.

Before and during the first hour after provocation, the FEV₁ was measured with the wet spirometer, as described for the histamine provocation procedure, as well as with a portable dry spirometer (micro-medical diary-card spirometer, Sensor Medics BV, Biltoven, The Netherlands). Thereafter, the subjects went home on the control day, and the FEV₁ was measured every hour in duplicate with the

Table 4. Pulmonary function test results

	Allergen	Δ FEV ₁ maximum early % baseline	Δ FEV ₁ maximum late % baseline	² log ratio PC ₂₀
1	Mite	-2.1	-9.6	-1.0
2	Mite	-8.3	-7.3	-0.2
3	Mite	-4.9	0.9	0.9
4	Mite	-6.2	-9.9	-0.8
5	Mite	-5.6	-0.01	1.0
6	Mite	-25.8	-8.5	-2.8
7	Mite	-8.2	-3.6	-2.2
8	Mite	-0.24	-1.1	0.6
9	Mite	-2.7	-10.1	-0.2
10	Mite	-19.4	-1.0	0.5
11	Mite	-7.7	-6.0	0
12	Mite	-8.9	-7.0	0.9
13	Cat	-10.4	-0.8	0
14	Cat	-4.8	-12.2	0
15	Cat	-10.8	-12.9	-0.5
16	Cat	-3.3	-4.8	-0.6
17	Cat	-8.1	-8.9	-1.8
18	Cat	-4.3	-10.1	-2.2
19	Cat	-6.4	-8.8	0
20	Cat	-23.8	-4.0	0
Mean (SEM)		-8.6 (0.22)	-6.3 (0.95)	-0.4 (0.2)

portable spirometer until the subject went to bed. Both spirometers were mutually calibrated. The next day, when the allergen was inhaled, the same procedure was repeated, but the patients stayed in the hospital for at least 12 h.

All FEV₁ values were expressed as percentage change from the baseline FEV₁ value (mean of three measurements within 5%), measured at the start of every study day. For each individual at each time point, the best FEV₁ value was corrected for diurnal variation according to the following formula:

$$\text{corrected } \Delta\text{FEV}_1 \text{ value (\% baseline)} = \text{FEV}_1 \text{ after allergen challenge (\% baseline)} - \text{FEV}_1 \text{ after control challenge (\% baseline)}$$

Statistical methods

Statistical methods included simple correlation, Student's *t*-test, and analysis of variance with linear trend analysis. Analyses were performed with the SPSS/PC+ statistical package (version 5.0) (Chicago, IL, USA). For statistical analysis, measurements below detection threshold were allotted half of the detection limit.

Results

Comparison of allergy tests with cat dander and *D. pteronyssinus* performed during the study period showed a percentage agreement between the routine RAST and skin test of 95% (380/400) in patients with an intracutaneous skin test threshold below 0.1 BU/ml. In patients with intracutaneous skin threshold above 0.1 BU/ml the percentage agreement was only 45% (107/240).

The results of the different allergy tests to characterize the patients are given in Tables 2 and 3.

The seven moderately sensitized allergic patients reacted to lower allergen concentrations in the skin test (geometric mean intracutaneous skin test threshold 0.017 BU/ml, prick test 10 000 BU/ml: mean wheal 7.2 mm, mean flare 31.2 mm). The results of the prick test with histamine 10 mg/ml were similar to those of the study group (mean wheal, respectively, 6.2 and 6.8 mm, mean flare 28.2 and 26.8 mm).

Four subjects of the study group had a negative RAST in the three specific IgE assays. Two of these four subjects had a positive histamine release from basophils with the relevant allergen, one subject was a nonresponder on all

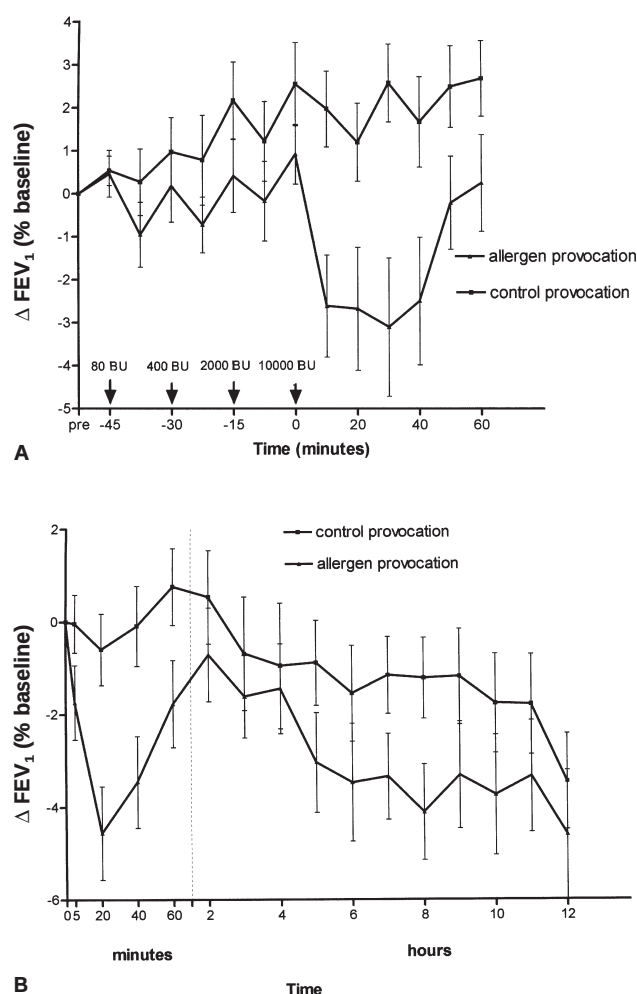


Figure 1. Results of bronchial allergen challenge for subjects with low levels of allergic sensitization ($n=20$, mean and SEM). A) Spirometric data obtained during up dosing of allergen and first hour after final allergen dose (10 000 BU/ml) and during control challenge as measured with wet spirometer. B) Spirometric values obtained with portable spirometer for 12 h after control challenge and allergen challenge.

stimuli, and in one subject, histamine release was not performed.

The positive control patients had significantly higher *in vitro* test results; mean IVR RAST 12.1 kU/l, mean routine RAST 12.8 kU/l, geometric mean total IgE 363.4 kU/l, histamine-release threshold 0.6 BU/ml. Geometric mean of the bronchial histamine threshold (4.2 mg/ml, GSD 2.7) did not differ significantly from the study group (7.9 mg/ml, GSD 3.3). The IVR RAST correlated well with the routine RAST ($r=0.73$, $P<0.0001$). Two subjects (nos. 16 and 18) had striking differences between the routine and the IVR RAST. Both subjects had a low or negative cat-dander routine RAST, while the IVR RAST showed a high RAST score.

Fig. 1 shows the results of the allergen challenge in the study group. All subjects tolerated the highest inhaled

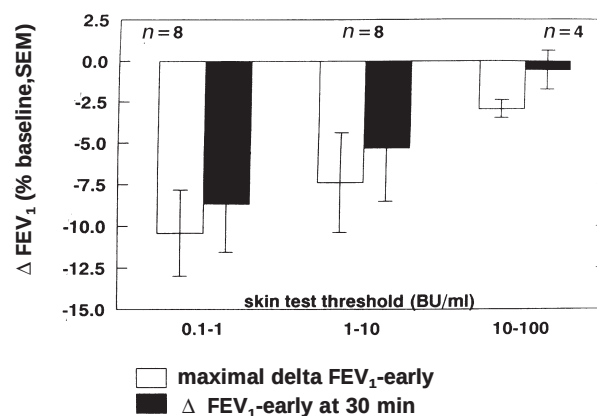


Figure 2. Relation between skin test threshold and early decrease in FEV₁ in subjects with low levels of allergic sensitization. Early decrease in FEV₁ is shown as maximal Δ FEV₁ in first hour and as Δ FEV₁ at 30 min.

allergen concentration (10 000 BU/ml). Only three of them showed a classical early response with a decrease of more than 15% in FEV₁ in the first hour after allergen challenge. None showed more than 15% decrease after 4–8 h. There was a statistically significant decrease ($P<0.005$) in FEV₁ in the first hour after challenge, on the time points of maximal drop in FEV₁ in the positive control group. The decrease remained significant after exclusion of the three subjects with a more than 15% decrease in the first hour ($P<0.005$). Concerning the late-phase reaction in this group, there was also a significant decrease ($P<0.05$) in FEV₁ expressed as mean decrease or area under the curve 4–8 h after allergen challenge.

The seven moderately sensitized allergic patients in the positive control group showed more than 15% decrease in FEV₁ in the first hour after challenge. The threshold was reached with less than 10 000 BU/ml in four of the seven patients. These four patients also had a classical late-phase response with more than 15% decrease in FEV₁ 4–8 h after challenge.

In the study group, a relationship was found between the skin test threshold and the early decrease in FEV₁ (linear trend, $P=0.046$) (Fig. 2).

Fig. 3 shows the change in PC₂₀ after allergen challenge in the study group. The results indicated a fall in PC₂₀ histamine after allergen challenge, from 9.1 to 6.7 mg/ml histamine, which did not reach statistical significance (paired Student's *t*-test, $P=0.09$). In the seven moderately sensitized patients, a clear increase in nonspecific bronchial responsiveness after allergen challenge was seen: from 4.2 to 0.9 mg/ml histamine (paired Student's *t*-test, $P=0.008$).

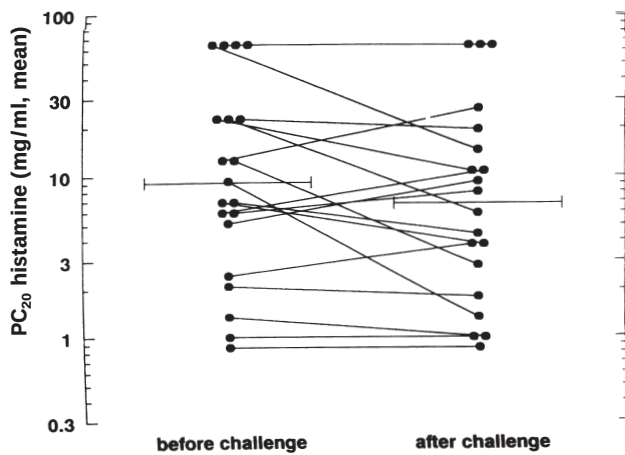


Figure 3. Change in PC₂₀ after allergen challenge for subjects with low levels of allergic sensitization.

Within the study group, there was no difference in early and late response between asthmatics and nonasthmatics. Moreover, no difference was found between patients challenged with house-dust mite and cat dander.

Individual results of the bronchial allergen challenge test are shown in Table 4.

Discussion

In contrast to the seven moderately sensitized allergic patients, only 3/20 patients with low skin reactivity showed a classical early-phase broncho-obstructive response after challenge with high-dose cat-dander or house-dust-mite allergen. This was not due to a significant difference in nonspecific bronchial hyperreactivity between the two groups.

For the group of subjects with low-grade allergic sensitization to indoor allergens, we found a significant early and late decrease in FEV₁, even when the three early-phase responders were excluded. Comparison of spirometric values between control day and challenge day on the five time points of maximal decrease in FEV₁ provided by the positive control group minimized bias. Although there was no formal investigator and subject blinding, we believe that the time course of the postchallenge reactions, the return to baseline values 2 h after challenge, and the relationship between skin reactivity and maximal Δ FEV₁ in the study group further exclude study bias.

Within this group, there was a relationship between skin test threshold and decrease in FEV₁. There was a trend toward decrease in bronchial histamine threshold. This indicates that even in patients with a low skin

reactivity, pathophysiologic reactions leading to airway obstruction and possibly bronchial hyperresponsiveness may occur after inhalation of allergen. Most patients will not be aware of a 5% reduction in FEV₁, which is the same order of magnitude as the diurnal variation in this group. Moreover, a 5% change in FEV₁ cannot be easily detected by spirometry in an individual patient, due to the imprecision of the test. This does not necessarily imply that small changes in lung-function parameters after allergen exposure are irrelevant to airways disease. This study has shown that even small (5%) early reduction in FEV₁ after allergen challenge is accompanied by a late-phase reaction and possibly by induction of nonspecific hyperresponsiveness. There is overwhelming evidence of a relation among late-phase reactions, nonspecific hyperresponsiveness, and airway inflammation (26–29). Beasley et al. took bronchial biopsies of patients with mild clinical and subclinical asthma and found clear indications of inflammatory changes (30).

In the patients with low levels of IgE antibodies, there is considerable variation between the results of the different RAST assays. Only 10 of the 20 subjects with an intracutaneous skin test threshold above 0.1 BU/ml were positive in all three RAST systems.

Four of the 20 patients with a positive skin test had a reproducible negative RAST in all three systems. The skin test in these patients was repeated and the same positive results were obtained. At least two of these patients had a positive histamine release from washed leukocytes with the allergen. This is in agreement with our earlier study on histamine release tests in 35 patients with a positive skin test but without detectable circulating IgE antibodies. For 80% of these patients, a positive histamine release test was obtained, strongly suggesting an IgE-mediated reaction and false-negative RAST results (3).

Two subjects had a negative cat routine RAST and a highly positive IVR RAST. Although the IVR RAST score was similar to the RAST scores of the positive controls, both subjects had skin test thresholds above 0.1 BU/ml and less than 15% decrease in FEV₁ after allergen challenge. All positive controls with such RAST scores showed a more than 15% decrease in FEV₁. The IgE antibodies of these two patients were not directed against the major allergen of cat dander, Fel d 1 (results not shown). The identity of the allergenic component with an apparently low biologic activity is currently under study.

In conclusion, we found in subjects with low levels of allergic sensitization, a small but significant early

and late decrease in FEV₁ and a trend for a decrease in PC₂₀ after allergen challenge. This indicates that patients with low-grade IgE responses to indoor allergens develop asymptomatic early and late bronchial reactions upon allergen exposure. This may lead to enhanced airway inflammation and nonspecific bron-

chial hyperresponsiveness in the absence of acute airway symptoms.

Acknowledgments We thank Dr J. A. Weber for performing the Pharmacia-CAP test. J. S. van der Zee was financially supported by The Netherlands Asthma Foundation.

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