

Original article

Early and late phase asthmatic response in lower airways of cat-allergic asthmatic patients – a comparison between experimental and environmental allergen challenge

Background: Standardized experimental allergen challenges are usually adopted to investigate the effect of allergen exposure on the lower airways. Environmental (natural) allergen challenges are used less often, mainly because of difficulties in standardizing the method, safety reasons and costs. The aim of this study was to investigate the relationship between an experimental and an environmental bronchial challenge. For this reason a natural challenge model was developed.

Methods: Sixty-two patients with a history of cat allergen-induced symptoms involving the lower airways, positive skin prick test, positive *in vitro* specific IgE to cat allergen and bronchial hyper-responsiveness were included. All 62 patients underwent an experimental challenge in the laboratory followed by an environmental allergen challenge.

Results: All 62 patients developed an early asthmatic response ($\geq 20\%$ fall in forced expiratory volume in 1 s (FEV_1)) in the experimental challenge and 60% (37/62) during the environmental challenge. A late asthmatic response ($\geq 15\%$ fall in FEV_1 within 3–24 h) was seen in 56% (35/62) of the patients after the experimental challenge. Following the environmental challenge 47% (29/62) of the patients developed a late response. Thirty-four per cent (21/62) of the patients developed a late response in both challenge models and 31% (19/62) did not develop a late response in any model. Thus, there was consistency in 65% (40/62) of the patients in both challenge models.

Conclusion: We found consistency in the pattern of response to inhaled allergen between the two challenge models and we believe that experimental bronchial challenge is likely to reflect the development of relevant inflammation in the lower airways after low-dose allergen exposure in the environment.

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Allergy to cat dander is the most common perennial allergy in Sweden and positive skin tests can be found in approximately 15% of the population aged 20–45 years (1). Many of these sensitized individuals are at risk of acquiring asthma symptoms upon cat allergen exposure (2, 3). As the number of cat-allergic individuals is large and cat allergen is widely distributed in the indoor

environment, this type of allergy has become a significant medical and social problem in the western world (4, 5).

Inhalation of allergen by sensitized individuals with a history of asthma results in bronchoconstriction that develops within 10–20 min and resolves within 1–2 h (early asthmatic response, EAR). In some individuals this immediate reaction is followed by a second phase of decreased lung function starting after 3–4 h, which may persist for 24 h or more (late asthmatic response, LAR) (6).

Late asthmatic response has often been used as an experimental allergic-asthmatic model of the inflammatory process because of its close resemblance to chronic asthmatic disease. The features of a patient's asthma that determine whether or not they develop LAR are unknown. It has been suggested that the level of allergen sensitivity (mirrored by skin prick test (SPT) responses

Abbreviations: BHR, bronchial hyper-responsiveness; EAR, early asthmatic response; EnBPT, environmental bronchial provocation test; ExBPT, experimental bronchial provocation test; FEV_1 , forced expiratory volume in 1 s; LAR, late asthmatic response; MPT, methacholine provocation test; PC_{20} , concentration of inhaled methacholine at 20% FEV_1 fall; PD_{20} , allergen provocation dose at 20% FEV_1 fall; SIT, specific immunotherapy; SPT, skin prick test; SQ, standard quality.

and amount of allergen specific IgE), allergen dose and the degree of bronchial hyper-responsiveness could be of importance (7–11).

Experimental challenges are usually adopted in asthma studies to investigate the effect of allergen exposure. Environmental (natural) exposure is used in pollen studies, but less often in perennial allergen studies because of difficulties in standardizing the method, safety reasons and costs. In an experimental challenge, high doses of allergen extract solution are nebulized and inhaled over a short-time interval. On the other hand, in an environmental challenge low amounts of allergen in particle form are dispersed in the inhaled air. The duration of an environmental exposure is longer and allergen deposition in the upper and lower airways differs from an experimental challenge (12, 13).

The aim of this study was to investigate, in the same cat-allergic asthmatic patient population, the occurrence of EAR and LAR in two different challenge models – the experimental and the environmental. In the experimental challenge, we used a dosimeter nebulizer for administration of allergen and in the environmental challenge model a domestic environment inhabited by cats was used.

Methods

Patients

The study included 62 patients, 24 men and 38 women, of mean age 31, range 18–46. All patients had a history of cat allergen-induced symptoms in the upper and lower airways. For inclusion, patients were required to have a positive SPT to cat allergen of at least 3 mm in diameter (Soluprick® SQ; Alk-Abelló, Hørsholm, Denmark), *in vitro* specific IgE to cat allergen of at least class 2 CAP FEIA (Pharmacia Upjohn, Uppsala, Sweden), methacholine PC₂₀ < 8 mg/ml and a positive allergen bronchial provocation test to cat allergen (at least 20% fall in FEV₁ at ≤ 64 000 standard quality (SQ/ml) of allergen concentration, Aquagen® SQ; Alk-Abelló). The patients had an intermittent asthma according to Global Initiative for Asthma (GINA) guidelines.

In addition to cat allergy 28 patients were also sensitized to birch and grass pollen, 57 to dog allergen and 43 to horse allergen. None of the patients had any pets at home. All patients had a negative SPT and negative clinical history to house dust mite and mould. None of the patients had been previously treated with inhalation steroids or allergen-specific immunotherapy.

The study was performed with the approval of the ethics committee of Göteborg University. Informed consent was obtained from the patients.

Study design

Patients fulfilling the inclusion criteria including positive experimental bronchial challenge underwent environmental allergen challenge. For practical reasons the environmental challenge was performed before the experimental challenge in 17 patients. Those patients were included in the study after positive results on experimental challenge. There was no difference in any parameter between this subgroup and the other patients. The time period between challenges was a minimum of 2 weeks. If patients were sensitized to seasonal allergens the provocations were performed out of season.

Skin prick test

All patients were tested out of season with a standard allergen panel consisting of birch, timothy, mugwort, dog, cat dander, house dust mite (*Dermatophagoides pteronyssinus*) and mould (*Cladosporium*). Diluent as a negative control and histamine chloride (10 mg/ml) as a positive control were also applied.

Methacholine provocation test

Methacholine chloride solution was nebulized in a Pari Inhalerboy (Paul Ritzau Pari Werk, Sternberg, Germany) nebulizer (output 0.8 ml/min with continuous nebulization) and inhaled by tidal breathing for 2 min. Methacholine was inhaled in doubling concentrations starting with 0.03 mg/ml to a maximum concentration of 16 mg/ml. The forced expiratory volume in 1 s (FEV₁) was measured 30 and 90 s after each inhalation. The provocation was continued with 5 min intervals between inhalations until a fall in FEV₁ of 20% or greater was obtained (14). The concentration of methacholine producing a 20% fall in FEV₁ (PC₂₀) was calculated by linear interpolation on a log-dose–response curve. Treatment with inhaled short-acting β_2 -agonists was not permitted 6 h prior to challenge. None of the patients was treated with theophyllines, long-acting β_2 -agonists or steroids.

Experimental bronchial provocation test

The experimental bronchial provocation test (ExBPT) was performed using a dosimeter nebulizer (Spira® Dosimeter; Spira Respiratory Care Center LTD, Hämeenlinna, Finland) with controlled tidal breathing (15). An aqueous solution of cat allergen extract (Aquagen® SQ, ALK-Abelló) was used for the test. A maximum of four concentrations, 1000, 4000, 16 000 and 64 000 SQ allergen units/ml solution was given with a initial dose of 13.75 SQ up to a maximum cumulative dose of 7026 SQ. After an initial lung function test (FEV₁), the patient inhaled diluent (four breaths). The FEV₁ was measured and designated as the 100% FEV₁ value. After each concentration of nebulized allergen, two registrations of FEV₁ were performed at 5, 10 and 15 min after inhalation and the highest value at each time point was recorded. The allergen provocation was continued with 15-min intervals between increased doses until a fall of ≥20% in the FEV₁ was obtained. The result was defined as the PD₂₀ (cumulative dose of allergen producing a 20% decrease in FEV₁). The registration of FEV₁ was performed 20, 30, 40 and 60 min following the time point of the 20% fall in FEV₁ and thereafter hourly. After 6 h, the patient left the clinic, but continued monitoring the FEV₁, recording hourly using a hand spirometer (One Flow Tester Memo, STI, Saint Romans, France) until bedtime. The final FEV₁ measurement was registered the following morning approximately 24 h after the challenge. The patients were allowed to use short-acting β_2 -agonists if the FEV₁ fall was > 20% during LAR. The effect of β_2 -agonist medication was corrected by excluding FEV₁ values measured during the 5 h following administration.

Late asthmatic response was defined as a fall in FEV₁ of at least 15% during the 3–24 h following the EAR.

Environmental bronchial provocation test

The environmental bronchial provocation test (EnBPT) was performed in the sitting room of a family house in which three cats have lived for more than 10 years. On four occasions before the start of provocation settled dust was collected from furniture surfaces by vacuuming for 5 min and the concentration of cat

allergen (Fel d1) was later determined. The concentrations of dog (Can f1) and house dust mite allergen (Der p 1) in settled dust were measured as well.

On each of the total of 22 provocation days, the concentration of airborne cat allergen in the provocation room was measured during the 3-h procedure. Air samples were obtained by using IOM samplers (SKC Inc., Blandford Forum, UK) equipped with 25 mm mixed cellulose ester filters, of pore size 0.8 μ m (Millipore Corp., Bedford, MA, USA). An air-flow rate of 2 l min over the filter was obtained using Gil-Air Personal Air Sampler pumps (Gilian Instrument Corp., Clearwater, FL, USA). On each provocation day two pumps were used and attached to the protective clothing in the breathing zone of two participants.

Prior to the EnBPT, patients were advised to avoid contact with cats and other pets to which they were sensitized for at least 1 week and they should have a FEV₁ > 60% of predicted value before the start. Using a hand spirometer, spirometry was performed and baseline FEV₁ values were recorded before patients entered the provocation room. On each provocation day, one to five patients participated and the procedures were monitored by a doctor and two study nurses. The provocations started in the morning (the room had not been used since the evening before). During provocation, ventilation was turned off, but a small domestic fan situated at a fixed place on the floor was used to spread the allergen in the air. One cat moved freely in the room about 20% of the provocation time (10 min/h). The patients moved around in a systematic way every 15 min. Forced expiratory volume in 1 s measurements were repeated every 15 min and the provocation was terminated when the FEV₁ had fallen by $\geq$ 20%. Ocular and nasal symptoms were recorded as well. The patient was then moved out of the room and inhaled 0.8 mg salbutamol. If the patient did not obtain a fall in FEV₁ of 20% the provocation was ended after 3 h. The patient was then moved out of the room, but obtained no medication. After provocation the FEV₁ was registered hourly until bedtime. The next morning, approximately 24 h after start of provocation, the last FEV₁ values were recorded. The patients were allowed to use short-acting β_2 -agonists if the FEV₁ fall was >20% during LAR. The effect of β_2 -agonist medication was corrected by excluding FEV₁ values measured during the 5 h following administration. Late asthmatic response was defined as a fall in FEV₁ of $\geq$ 15% up to 24 h following provocation.

Sample extraction and Fel d1 assay

The settled dust samples were sieved through a 0.3-mm mesh to produce a fine dust. A 50-mg aliquot was stored at -20°C until extraction. The filters containing airborne dust were placed in a test tube and kept at -20°C until extraction. Extraction was performed with 10 ml of phosphate-buffered saline (pH 7.4) containing 1% bovine serum albumin (BSA) and 0.05% Tween 20 by rotation overnight at 4°C . The extracts were analysed using a two-site enzyme linked immunosorbent assay (ELISA) method (16).

Calculation of inhaled allergen dose during ExBPT

The cumulative inhaled Aquagen dose in SQ during provocation for each patient was multiplied by 0.15 (1 SQ corresponds to 0.15 ng Fel d1 according to manufacturer) to determine the dose in ng.

Calculation of inhaled allergen dose during EnBPT

The allergen concentration in the air during provocation in ng/l was multiplied by the patient's tidal volume (calculated to 0.5 l).

This value was multiplied by the patient's breathing frequency/min and the number of minutes the provocation lasted. The calculated dose is approximate since the particle size and hence the respirable fraction was not determined in this study (12).

Statistics

The Mann-Whitney *U*-test for nonpaired data was applied. A *P*-value of <0.05 was considered statistically significant.

Analyses were performed using Statview software (SAS Institute Inc., Cary, NC, USA).

Results

Experimental bronchial provocation test

Early phase response. All sixty-two patients developed an EAR ($\geq$ 20% fall in FEV₁) following experimental allergen bronchial challenge. The median value of maximal fall in FEV₁ was 26%, range 20–63. The median value of allergen PD₂₀ was 342.9 SQ, range 13.75–2301.6 and the median inhaled dose of allergen/patient during provocation was 63.9 ng, range 2.1–525.9.

Late phase response. Fifty-six per cent (35/62) of the patients developed a LAR ($\geq$ 15% fall in FEV₁). If the patients who had a fall in FEV₁ of between 10% and <15% were included, the proportion of patients with a late response increased to 71% (44/62) of the patients. The median time point of maximal fall in FEV₁ was 9 h following PD₂₀ time point, range 4–24. For all 62 patients participating in the study, the median value of maximal fall in FEV₁ at 3–24 h following challenge was 15%, range 0–45.

Environmental bronchial provocation test

High levels of cat antigen were measured on furniture surfaces in the provocation room (median value 852 000 ng Fel d1/g dust), but concentrations of dog and house dust mite allergen were found to be low (2180 ng/g dust for dog allergen and <100 ng/g dust for mite allergen).

Early phase response. Sixty per cent (37/62) of the patients developed a fall of $\geq$ 20% in FEV₁ during the time interval that they were exposed to allergen in the environmental challenge. The median time point for EAR was 120 min, range 20–180 min. For the other 25 patients who did not develop an EAR, the value of median maximal fall in FEV₁ during the 3-h provocation was 10%, range 2–19. For all 62 patients in the study the median maximal fall in FEV₁ during early phase was 20%, range 2–37. No clinically severe early reactions were seen. For inducing ocular and nasal symptoms the median time was 30 min, range 15–180. The median allergen concentration in air during the 22 provocation

days was 13.1 ng/m³, range 5–28 and the median inhaled dose of allergen/patient during provocation was approximately 13.9 ng, range 1.5–40.3.

Late phase response. Forty-seven per cent (29/62) of the patients developed a LAR. If the patients with a fall in FEV₁ of between 10 and <15% were added, the group increased to 71% (44/62) of the patients. The median time point of maximal fall in FEV₁ following provocation was 9 h after allergen challenge, range 5–24. For all 62 patients participating in the study, the median value of maximal fall in FEV₁ at 3–24 h following provocation was 15%, range 2–43. No clinically severe late reactions were recorded.

Comparison of ExBPT and EnBPT

When the sixty-two patients were divided with regard to the development of a LAR in both models, four groups could be discerned: group 1 consists of patients with a LAR in both challenge models ($n = 21$), group 2 consists of patients who developed a LAR after the experimental challenge, but not the environmental ($n = 14$), group 3 consists of patients who developed a LAR after the environmental challenge, but not the experimental ($n = 8$) and group 4 consists of patients without a LAR in both challenge models ($n = 19$). The distribution of patients with a LAR following the experimental and natural challenges and a comparison between different parameters is shown in Table 1.

Thirty-four per cent (21/62) of the patients were LAR-positive and 31% (19/62) were LAR-negative in both models. Thus, there is consistency in development of LAR in 65% (40/62) of the patients (Fig. 1). If the patients with a fall in FEV₁ of between 10% and <15% in the remaining 22 patients were added the consistency reach 74%.

Sixty per cent (21/35) of patients developing a LAR in the experimental challenge also developed a late response in the environmental challenge and 72% (21/29) of patients developing a late response in the

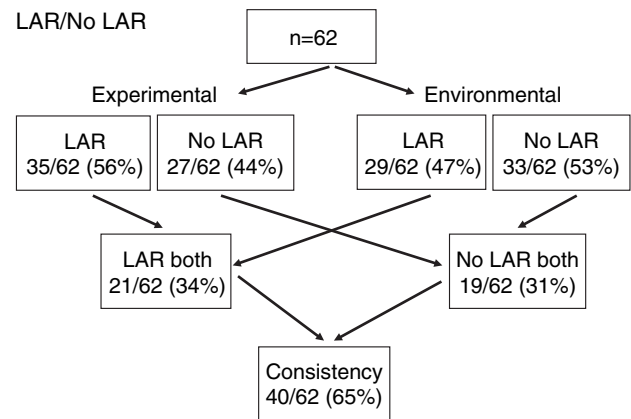


Figure 1. Distribution of development of a late asthmatic reaction (LAR vs no LAR) in the experimental and environmental challenge models. Patients with a fall in FEV₁≥15% were included.

environmental challenge also developed a late response in the experimental challenge. If the patients with a fall in FEV₁ of between 10% and <15% were included 82% (36/44) of the patients developed a LAR in both groups (Fig. 2).

While all patients on experimental challenge developed an EAR, only 60% (37/62) did so during the environmental challenge. However, nine of the 25 patients who did not develop an EAR in the environmental challenge developed a LAR. Five of these nine patients had a fall in FEV₁ close to 20% (median 18%, range 15–19) during the early phase.

Patients with a LAR in both challenge models showed significantly higher sensitivity to methacholine ($P = 0.03$) and had a significantly lower allergen PD₂₀ ($P = 0.005$) compared with patients without a LAR in any model (Table 1). No significant differences were seen between the two groups in SPT, specific IgE levels, eosinophils in serum or FEV₁% pred. There were no significant differences between patients in group 2 and those in group 3.

Table 1. Patients with LAR maximal fall in FEV₁≥15% = positive] and without LAR (maximal fall in FEV₁ <15% = negative) in experimental and environmental bronchial allergen challenge

Group	Experimental challenge	Environmental challenge	Number of patients	Methacholine PC ₂₀ (mg/ml), median (range)	Allergen PD ₂₀ (SQ-U/ml), median (range)	RAST (kU/ml), median (range)	SPT (mm ²), median (range)	Eosinophils in serum (10 ⁹ /ml), median (range)	FEV ₁ % pred. median (range)
1	Positive	Positive	21	0.2* (0.04–4.5)	155.5** (13.75–1310.4)	11.5 (3.5–75.2)	6.5 (4.0–17.5)	0.3 (0.1–0.6)	90.2 (71.3–110.0)
2	Positive	Negative	14	0.3 (0.04–2.1)	218.2 (13.75–2301.6)	8.1 (0.54–62.4)	8.5 (6.0–15.5)	0.2 (0.0–0.8)	94.7 (73.2–113.9)
3	Negative	Positive	8	0.9 (0.16–3.6)	110.0 (13.75–1160.6)	12.7 (1.5–22.0)	7.5 (5.5–9.5)	0.2 (0.1–0.3)	86.7 (70.7–98.0)
4	Negative	Negative	19	1.7 (0.06–7.6)	1115.2 (19.9–2299.4)	9.6 (1.2–41.9)	6.2 (4.0–17.5)	0.3 (0.1–0.7)	94.7 (68.1–109.8)

Patients with LAR in both challenge models (group 1) showed a significantly lower methacholine PC₂₀ (* $P = 0.03$) and a significantly lower allergen PD₂₀ (** $P = 0.005$) compared with patients with no LAR in any challenge model (group 4). No significant differences were observed between the two groups in the other parameters. FEV₁, forced expiratory volume in 1 s; LAR, late asthmatic reaction; PC₂₀, concentration of inhaled methacholine at 20% FEV₁ fall; PD₂₀, allergen provocation dose at 20% FEV₁ fall; SPT, skin prick test; SQ, standard quality; RAST, radioallergosorbent test.

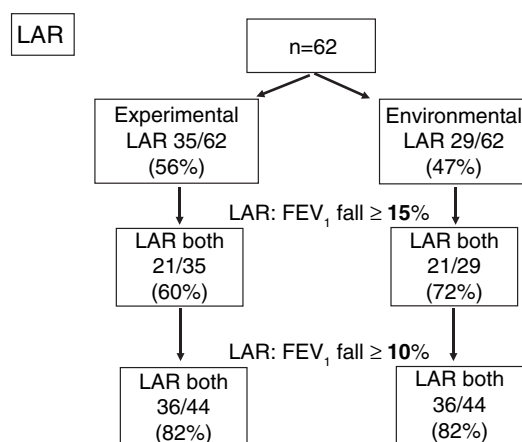


Figure 2. Distribution of development of late asthmatic reactions (LAR) in both experimental and environmental challenge models. Patients with a fall in FEV₁ of $\geq 15\%$ or $\geq 10\%$ respectively, were included.

Discussion

We have shown that around 65% of allergic asthmatic patients display a similar pattern of response (LAR or no LAR) to inhaled allergen in both the experimental and the environmental models of bronchial challenge.

The main differences between the two models are (i) the form of inhaled allergen (as a solution of allergen extract in ExBPT or as particles dispersed in the inhaled air in EnBPT), which also influences; (ii) deposition in the airways; and (iii) the time factor. The knowledge about the relationship between experimental and environmental allergen bronchial challenges is limited. Van Metre et al. (12) used a cat-exposure room to quantify the doses of cat allergen inhaled from ambient air required to induce a 20% fall in FEV₁. They found that these doses correlated with the doses of cat extract required to induce a 20% fall in FEV₁ in an experimental challenge model. However, the necessary doses were uniformly lower in the environmental challenge. Other studies have also reported that the doses required to induce symptoms during an environmental challenge were much lower when compared with those used in experimental challenge (17, 18). In an experimental challenge, a nebulized solution of allergen extract (drop size 2–5 μm) is inhaled during a short-time period and deposited mainly in smaller airways. Lieutier-Colas et al. (13) found that the allergen PD₂₀ obtained using an aerosol with a particle size of 10.3 μm (mainly deposited in proximal airways) was much lower than with aerosols with particles sizes of 1.4 and 4.8 μm , respectively (mainly deposited in smaller airways). In an environmental challenge, inhaled cat allergen represents a wide range of particle size and is therefore deposited in different parts of the airways including the proximal airways and is inhaled continuously during a longer period of time (12). This may be the

reason why a lower allergen dose could evoke EAR in the environmental challenge compared with standard experimental challenges where a nebulizer releases particles of 2–5 μm . In our study, larger allergen doses were also required to evoke an immediate fall in FEV₁ in the experimental challenge compared with the environmental challenge. Earlier studies have shown that in average 25% of the airborne allergen content in houses with cats were small particles < 5 μm (19). However, no particle size measurement was performed in our study.

The pollen seasons, for example birch, grass and ragweed seasons (20–22), are often used as a natural challenge model, while in perennial allergy environmental challenges are employed less often due to standardization problems, safety reasons and costs. Still, environmental challenge allows evaluation of both upper and lower airways and has been used in previous cat immunotherapy studies (23–25), as well as in other studies using a cat-exposure model (26–28). It is desirable, however, that the airborne allergen content is measured to ensure levels high enough to induce symptoms and facilitate comparison of allergen levels between provocations.

Commonly an EAR is defined as a reduction in FEV₁ of 20% following allergen challenge. In our study, patients in the experimental challenge inhaled relatively high doses of allergen and all developed an EAR (as this was inclusion criterion). In the environmental challenge the provocation was terminated after 3 h (for practical reasons) and only 60% of patients developed an EAR. Among 25/62 patients without EAR, nine developed a LAR. In 5/9 FEV₁ decrease was close to 20% and we believe that extended exposure would probably lead to further FEV₁ decrease. Our findings agree with results of an earlier study that showed an isolated LAR following a repeated low-dose allergen exposure (29). This is of clinical importance as the allergic individual may not always be aware of allergen exposure before onset of symptoms.

A LAR is usually defined as a fall in FEV₁ starting about 3–4 h and peaking after 6–12 h following allergen challenge. In both challenge models the median value for the time of maximal FEV₁ decrease was 9 h. In a few patients the maximal FEV₁ fall occurred between 12 and 24 h after the challenge. Nocturnal asthma and morning FEV₁ dips following a single allergen exposure have been previously reported (30). We therefore recommend that the recording of FEV₁ changes should be extended to at least 24 h.

There are few previous studies examining the development of a LAR following bronchial challenge with cat allergen. Mosimann et al. (31) found a LAR in eight of 21 patients (38%) after ExBPT with cat allergen and in another study Rohatgi et al. (32) demonstrated a LAR in four of seven patients (57%). In a much larger population of cat allergic asthmatics we have found a LAR following allergen challenge in about 50% of the patients in both ExBPT and EnBPT.

In 22 patients who developed a LAR in one challenge model, but not in the other, the data of some showed FEV₁ decreases close defined arbitrary cut-off point of 15%. As a consequence, patients below this cut-off point were excluded from the evaluation. In reality, there is probably a little difference between patients around the cut-off point. It is possible that a few more patients would have developed a LAR if exposed to higher doses of allergen. A number of studies in the literature indicate this possibility (33, 34).

The allergen-specific and nonspecific bronchial hyper-responsiveness seem to be the main factors behind the development of a LAR in our study, while SPT and allergen-specific IgE-levels are of less importance. It should however be emphasized that only patients with a rather high degree of allergen sensitivity (SPT and levels of specific IgE in serum) were included in the study.

In conclusion, an environmental allergen challenge is closer to the natural allergen exposure compared with allergen challenge in laboratory. It is however, difficult to perform, time consuming and requires special safety proceedings. Still, while consistency between both challenge methods is not complete our study indicates that an experimental challenge reflects reasonably well the allergen response in environmental conditions.

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