

Exposure chamber for allergen challenge. A placebo-controlled, double-blind trial in house-dust-mite asthma

Rønborg SM, Mosbech H, Poulsen LK. Exposure chamber for allergen challenge. A placebo-controlled, double-blind trial in house-dust-mite asthma.

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Exposure chambers have proven to be valuable tools in the study of reactions to aeroallergens, and in monitoring the efficacy of antiallergic therapy. In the present study, 15 house-dust-mite-allergic asthmatics and five nonallergic volunteers were challenged in a recently developed exposure chamber. The trial was performed double-blinded with house-dust-mite allergen or placebo. Patients with allergy to house-dust mite (*Dermatophagoides pteronyssinus*) (Der p) were included by positive skin prick test, allergen-specific IgE, and conventional bronchial allergen challenge, with nebulizer and mouthpiece. In the exposure chamber, a total allergen dose corresponding to 1200 ng Der p 1 was applied. All participants kept diaries, recording peak expiratory flow rates, symptoms, and medication in periods of at least 2 weeks before and after each challenge. Twelve of the 15 asthmatics reacted with asthmatic symptoms with a median change in FEV₁ of –16.4% when exposed to the allergen, but not to placebo, in the exposure chamber. Three patients had only minor symptoms during both chamber exposures and experienced no impairment of pulmonary function. Late-phase reactions were less frequent (one vs three) after the exposure chamber challenges, as compared to the traditional challenges. None of the healthy subjects reacted to the challenges. In conclusion, our exposure chamber was able to elicit symptoms in allergic subjects, and this ability was obtained with only minor amounts of house-dust-mite allergen. The described method could prove to be a more physiologically relevant model to monitor individual responses to aeroallergens.

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In the last decade, exposure chambers were introduced in allergology as a method to study the individual responses to known amounts of allergens in a controlled environment. The pioneer work by van Metre and Horak has contributed to our understanding of the immediate and late-phase responses to challenges with airborne allergens, as well as the differences in aerodynamic behavior of various native aeroallergens from cat, pollens, and house-dust mites (1, 2). The exposure chamber model also offers the means to monitor the effect and onset of action of antiallergic drugs at known allergenic loads (3–5).

We have previously developed an exposure chamber that fits in an existing room with very modest changes (6). The present study aimed to

evaluate further the concept in a placebo-controlled, double-blind trial, with challenge of asthmatic house-dust-mite-allergic subjects, and nonasthmatic volunteers. The trial included a comparison with results obtained from conventional bronchial challenges in the same patients. By using only minor allergen amounts, we wanted to mimic the naturally occurring allergenic levels in an effort to initiate symptoms in a more physiologic manner.

Material and methods

Patients

Fifteen (nine women, six men) house-dust-mite-allergic asthmatic patients and five (three women,

two men) nonallergic and nonasthmatic volunteers participated. The median age was 23.8 years (range: 18–38) in the group of house-dust mite allergics, and 31.8 years in the group of nonallergics (range: 24–48).

The patients included were allergic to the dust mite *Dermatophagoides pteronyssinus* (Der p), as defined by skin prick test with a mean diameter of ≥ 3 mm with Der p allergen solution, Soluprick®, 10 HEP (ALK, Hørsholm, Denmark); Der p allergen-specific IgE $\geq$ class 2, measured by two different methods, CAP® RAST (Pharmacia, Uppsala, Sweden) and Magic Lite® (ALK); and a positive basophil histamine release test (HR test) $\geq$ class 2 (Reference Laboratory, Copenhagen, Denmark). Allergic asthma was verified by a positive conventional challenge test with a Wright (Clement Clarke Int., Essex, UK) vial chamber nebulizer and mouthpiece with Der p allergen (Aquagen® SQ, ALK). A 3-ml volume was nebulized and inhaled for 2 min, at 10-min intervals. Lung function was measured at short intervals (2 min). A $\geq 15\%$ decrease in FEV₁ was considered positive, and terminated the challenge. Tenfold increases in allergen concentrations from 100 SQ-U/ml to a maximum of 100 000 SQ-U/ml were used. The bronchial sensitivity to allergen was expressed as the provocative dose (PD₁₅), calculated by interpolation in a semilogarithmic dose-response plot as the dose giving a fall in FEV₁ of 15% (7).

Design

The trial lasted 8 weeks or more for all subjects. Observation periods of at least 14 days with a stable lung function, defined as a day-to-day variation in peak expiratory flow rate (PEFR) of $\leq 15\%$, preceded and followed all challenges in the trial. All subjects visited the exposure chamber twice at 14-day intervals, receiving a challenge of placebo or allergen in random order (Fig. 1). All participants, allergic and nonallergic, kept diaries with daily records of PEFR (premedication values, best of three measurements), symptoms, and medication. Measurements were made twice daily (symptoms related to asthma and PEFR) or once daily (symptoms from eyes, nose, or other organs). In the 24 h following an exposure, at least six measurements were made. Patients collected mattress dust by vacuuming, using the ALK allergen-collecting nozzle with a micropore filter (8). The house-dust-mite allergen content was determined by ELISA for group 1 allergens, and the result was expressed in nanogram allergen per gram of dust. The detection limit of this analysis is 2 ng Der p 1/ml of solution. The content of three different house-dust-mite species was determined by this analysis

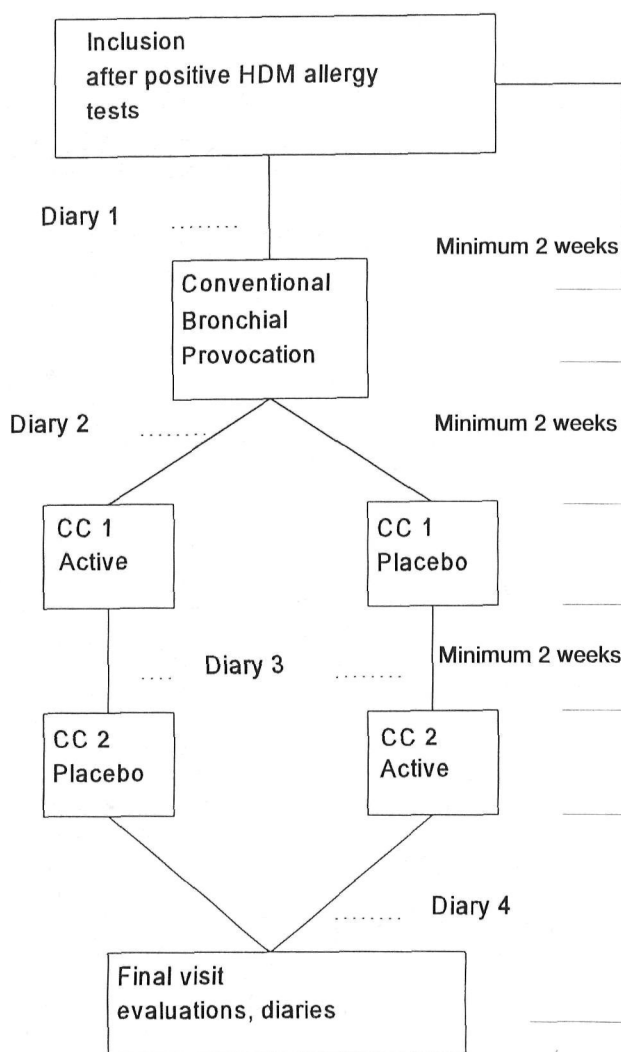


Fig. 1. Trial flowchart. Placebo or active allergen challenge in random order in chamber exposures. CC = chamber challenge; HDM = house-dust mite.

(*D. pteronyssinus*, *D. farinae*, and *D. microceras*) (Diagnosis Laboratory, ALK).

Allergen

The same batch of allergen from a monostrain culture of *D. pteronyssinus* containing all allergenic components was used in all challenges (kindly provided by Lisbeth Møller Christensen, ALK). The content of major allergen (Der p 1) was established by the ELISA method mentioned above. The allergen was divided into aliquots and stored in a 50% glycerol solution at -20°C until the final dilution on the day of challenge. Buffered saline (pH 7.3) with 0.3% human serum albumin (HSA) was used as diluent and for the placebo challenges. The solutions of allergen used were prepared by a person who did not

participate in conducting the challenges or patient monitoring.

Challenge in the exposure chamber

The participants were not allowed to take anti-histamines (topical or systemic) up to 2 weeks before any challenge (conventional or exposure chamber). Systemic steroid treatment was not allowed during the last month before challenge. Topical steroids were stopped for 48 h before challenge, short-acting β_2 -agonist for 8 h, and long-acting β_2 -agonist for 16 h. No other antiallergic drugs were used by the patients. As some patients avoided taking o.d. medication up to 96 h before challenges, the medication score data are presented as changes in actual o.d. doses of fast-acting β_2 -agonists.

Patients with pollinosis were challenged outside the pollen seasons. Each exposure chamber challenge session lasted for a maximum of 3 h. The patients were free to leave at any point during the sessions, and were also allowed to define the exact onset of an experienced positive bronchial reaction, without knowing the actual decrease in FEV_1 at that time. The lung function was monitored by measuring FEV_1 and FVC every 15 min with a compact electronic spirometer (Vitalograph MDI-Compact type 42.400, Buckingham, UK). PEFR was recorded with a Wright Mini-peakflowmeter at the same intervals, along with symptoms from eyes, nose, skin, and/or other organs, on a scale of 0–3 in symptom severity. At the end of a chamber challenge session, the subject made an evaluation of the overall experienced outcome by answering the question, "Did you experience any allergic reaction?", Yes or No. The patients were observed after the challenges until pulmonary function had stabilized and symptoms from other organs had disappeared.

The chamber has previously been described in detail (6). The allergen/placebo solution was nebulized into the air inlet (4000 l/h) in four sequences of 10 min at 2-min intervals, each challenge being initiated with the equipment described previously (6). The amount of the major allergen Der p 1 in the extract nebulized was 1200 ng. After each trial, the residual volume of allergen solution left in the nebulizer was measured and stored at -20°C for later analysis. Allergen was collected on glass EPM 2000 microfiber filters (pore size $0.6\ \mu\text{m}$) (Whatman®, Maidstone, UK) mounted on a high-volume Sartorius MD 8 air sampler (Sartorius GmbH, Göttingen, Germany). The airflow in the chamber was balanced by setting the sample rate at 4000 l air/h. This corresponded to an air-exchange rate of 0.34/h (total volume of chamber:

11.8 m³). The filters were changed at 45-min intervals.

The trial subjects followed an occupancy pattern with 2 min of standardized activity every 15 min inside the challenge chamber, to ensure standardized air mixing, i.e., turbulent airflow in the chamber, during the experiments. Challenges were performed at ambient humidity and temperature. However, for the comfort of the participants, and for uniform nebulizing conditions, the humidity was partially controlled in range (i.e., kept at $\leq 10\%$ above ambient relative humidity). This was achieved by using a HETOFRIG (prototype) dehumidifier, mounted inside the challenge chamber (Heto Holten A/S, Copenhagen, Denmark). The temperature was monitored by a dual electronic thermometer, Hanhart Memotherm (Hanhart, Germany) with sensors mounted both inside and outside the challenge chamber. Humidity was measured by an electronic hygrometer (Omron, Tokyo, Japan).

Statistics

A computer program, Sigmastat version 1.0 for Windows, was used (Jandel Scientific GmbH, Erkanth, Germany). The Mann–Whitney U-test was used to compare the decreases in FEV_1 and changes in PEFR, medication, and symptoms; the Wilcoxon test was used to compare paired data. The *t*-test was used to detect any priming/periodical effect caused by the order in which the placebo/allergen challenge in the exposure chamber was conducted. Two-tailed *P* values of ≤ 0.05 were considered significant.

Safety and ethics

The patients were closely monitored by a physician during and after all challenges. Rescue medication was at hand, along with equipment for resuscitation. The patients were observed by the same physician after the challenges and were given written and oral instructions on possible late-phase reactions after the trials. All patients were equipped with oral steroid tablets and fast-acting β_2 -agonist inhalation medicine for emergency use.

The trial was performed in accordance with the Helsinki Declaration, and was approved by the local ethics committee.

Results

The demographic data on all patients and healthy subjects, the results from all challenges, conventional and in the exposure chamber; and concentrations of mite allergen in mattress-dust samples are shown in Table 1. Acute reactions to the

Table 1. Patient data and outcome of allergen challenges and collections

Patient no.	Sex Age (years)	PD ₁₅ SQ-U (in cbc)	Order of chamber exposures	Patient evaluation of allergic reactions	Symptoms Other organs in active chamber challenge	ΔFEV ₁ in active chamber challenge	Der p 1 ng/g of mattress dust
01	M 28	75 000	AP	-/-	+ n, s	-6.8	2819
02	F 22	325	PA	-/+	lpr (acc)+e, n	-23.6	104
03	M 23	50 000	PA	-/+	lpr (cbc)+e, n	-6.2	36
04	M 23	90 000	PA	-/+	+e, n	-12.3	68
05	M 23	32 000	AP	+/-	+e, n, s	-10.1	2175
06	F 25	23 000	PA	-/-	+n	-7.8	52
07	M 24	35 000	PA	-/+	+e, n	-28.1	432
08	M 31	55 000	AP	+/-	lpr (cbc)	-20.1	263
09	F 21	5500	PA	-/+	lpr (cbc)	-26.6	2539
10	M 25	2800	AP	+/-		-15.4	122
11	M 30	4800	PA	-/+		-13.7	28 207
12	F 24	3200	AP	+/-	+s	-28.4	4085
13	F 38	4800	PA	-/+		-12.8	9755
14	F 18	7000	AP	-/-	+n	-8.1	230
15	M 20	10 000	AP	+/-	+e	-17.4	6382
25	M 23	Neg	AP	-/-		> -5	n.d.
26	F 31	Neg	PA	-/-		> -5	n.d.
27	M 27	Neg	AP	-/-		> -5	n.d.
28	M 29	Neg	AP	-/-		> -5	n.d.
29	F 48	Neg	PA	-/-		> -5	n.d.

Demographic data and outcome of challenges. lpr=late-phase reactions, cbc=conventional bronchial challenge, acc=active chamber challenge, e=eyes, n=nose, s=skin, n.d.=not done, A=active, P=placebo.

exposures are shown in Figs. 2–4, while late responses are illustrated in Fig. 5A and B. The patients were divided into two groups, according to their sensitivity (expressed as PD₁₅) in the conventional bronchial challenge. Each group is presented with its own symbol in Figs. 2–5A and B.

Twelve of 15 patients reported bronchial symptoms in response to the active challenge in the exposure chamber; accordingly, this challenge was correctly evaluated as positive. These twelve patients had a median change in FEV₁ of -16.4% (range -6.2 to -28.4) compared with a median of -13.7% in all patients. The remaining three patients did not experience bronchial constriction in any of the chamber challenges; however, two of them responded to both challenges in the chamber with a few symptoms from other organs, but evaluated both exposures as negative. None of the five healthy volunteers reacted to any of the challenges (Table 1).

One patient reactive to a very low allergen dose in the conventional bronchial challenge suffered a moderate (ΔPEFR -22%) late-phase reaction (with onset 8 h after, and a duration of approx. 1 h) after the active exposure chamber challenge, whereas three other patients, all with intermediate sensitivity, suffered late-phase reactions after the conventional bronchial challenge (Table 1).

The acute pulmonary responses to all challenges are presented in Fig. 2. The reactions to the placebo

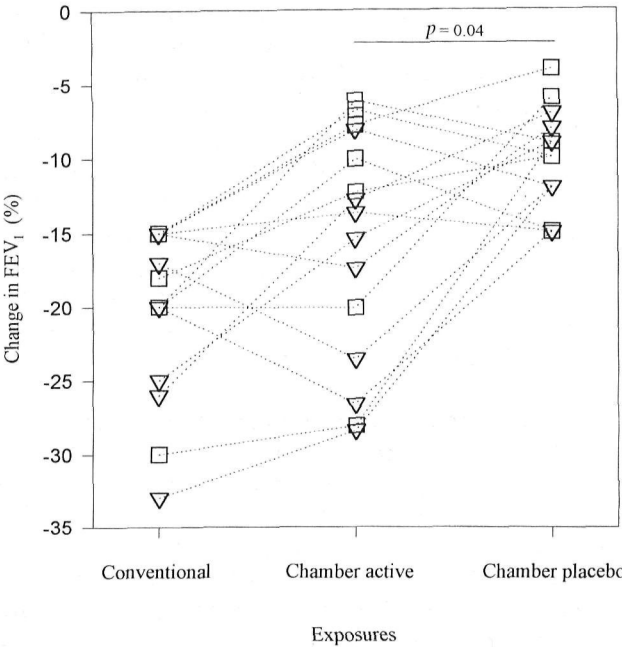


Fig. 2. Acute responses in lung function to three different challenges. Symbols: highly and median sensitive: ▽ (PD₁₅ ≤ 10 000 SQ-U), less sensitive: □ (PD₁₅ ≤ 100 000 SQ-U).

chamber challenges were significantly lower than the reactions recorded in the active chamber challenge ($P=0.04$). Four patients (nos. 1, 3, 6, and 14 in Table 1) had a more pronounced decrease in

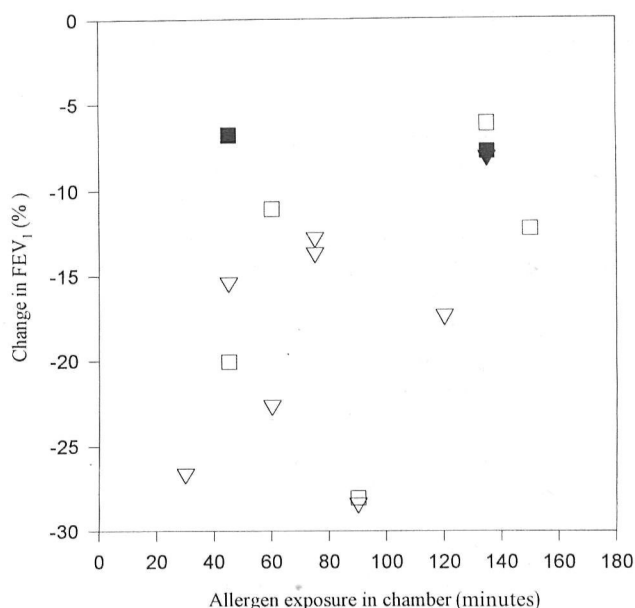


Fig. 3. Acute response in lung function to allergen challenge in exposure chamber. Duration of allergen exposure in minutes to maximum fall in FEV₁ occurred/or patient chose to leave chamber due to symptoms (filled symbols). Main symbols as in Fig. 2. No linear correlation was found ($r=0.39$; $P=NS$).

FEV₁ in response to the placebo than to the active challenge. However, one patient (no. 3 in Table 1) in this group correctly identified the positive allergen challenge due to eye and nose symptoms and slight tracheal irritation.

Fig. 3 shows the time of allergen exposure in the challenge chamber before the maximum decrease in FEV₁ occurred, or the patient chose to leave the chamber due to symptoms. No linear correlation was observed between these two parameters ($r=0.39$; $P=NS$). Similarly, a separate analysis of the subgroup of patients staying in the chamber for the full challenge period did not disclose a correlation ($r=0.43$; $P=NS$).

The provocative dose (PD₁₅) in the conventional bronchial challenge is shown against time to maximum decrease in FEV₁ in response to the active chamber challenge in Fig. 4. There was no linear correlation between the two parameters ($r=0.22$; $P=NS$). Filled symbols represent the seven patients with the highest allergen levels in their mattress dust samples (>2000 ng Der p/g mattress dust) (Table 1). Six out of seven reached their maximal decrease in FEV₁ within 90 min in the active chamber challenge, and four of these patients had a high sensitivity in the conventional bronchial challenge (PD₁₅ $\leq 10\,000$ SQ-U).

The diary recordings 96 h in advance of, and 24 h after, each challenge (conventional or challenge chamber) were used to calculate the mean PEFR values. The results are shown in Fig. 5a. Changes

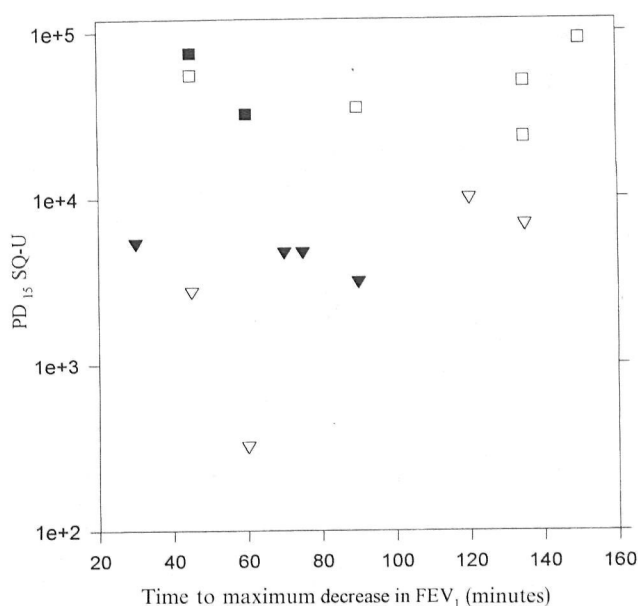


Fig. 4. Acute response in lung function. PD₁₅=provocative dose in conventional challenge (SQ-U) shown against time to maximum decrease in FEV₁ in active chamber exposure. No linear correlation was found ($r=0.22$; $P=NS$).

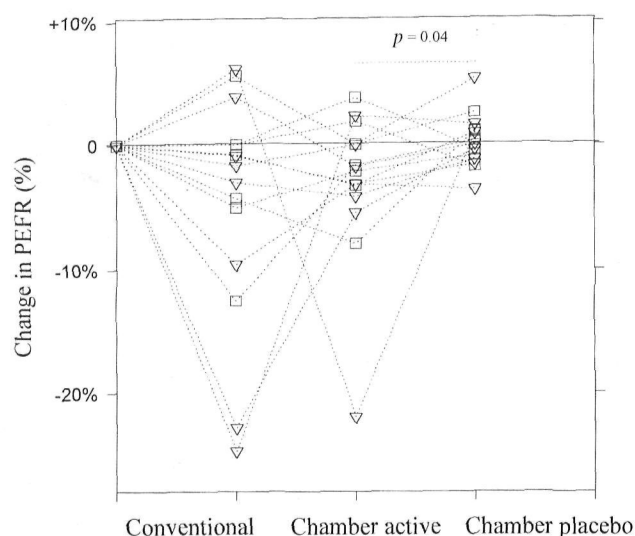
in mean PEFR were significantly lower after the placebo challenge in the exposure chamber than after the active exposure chamber challenge ($P=0.04$).

No significant difference was seen when comparing the effect of the different exposures on medication with o.d. fast-acting β_2 -agonist and symptom scores (change from mean value 96 h before to mean value 24 h after challenges) (Fig. 5b).

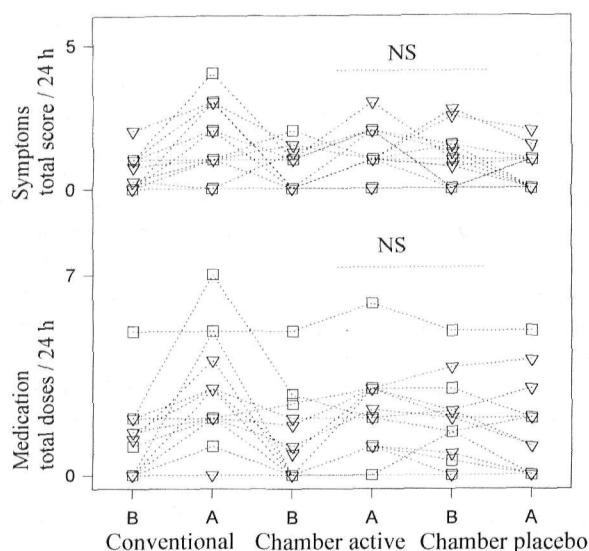
Discussion

By using an exposure chamber, we have studied the allergic reaction to a known allergenic load. In our chamber concept, we used nebulized allergen in four sequences to mimic the natural aerodynamic behavior of house-dust-mite allergen, as described in previous works by Sakagushi et al., Platts-Mills et al., Wood et al., and De Blay et al. (9–12). House-dust-mite-allergic asthmatics were chosen as participants in this study because of the major role this allergic disease plays in the West (13–17). The allergen sampling and analysis methods applied in our exposure chamber concept were similar to those used in a number of previously published studies on aeroallergens (18–25).

We chose to design our trial as a placebo-controlled, double-blind study, and included healthy volunteers to compare allergen-specific and non-specific or psychologic factors as the cause of experienced symptoms. Twelve of 15 patients experienced



(a) Types of exposures



(b) Types of exposures

Fig. 5a) Late responses in lung function to three different challenges. Mean PEFR during 24 h after challenge in percentage of mean PEFR during 96 h before exposures. b) Late responses after exposures as registered in medication and symptom scores. Mean medication (actual doses o.d. of fast-acting β_2 -agonist/24 h) calculated 96 h before and 24 h after exposures. Total symptom scores calculated likewise. Symbols as in Fig. 2.

allergic symptoms in various degrees in response to the active challenge in the exposure chamber, and none of the five healthy volunteers reacted to two full-length stays each of 3 h in the exposure chamber. The results show that our concept is applicable and safe when applied to a group of house-dust-mite allergics. For safety reasons, the patients were allowed to leave the exposure

chamber and to initiate treatment at any point. This fact might in some cases explain why a decrease in FEV_1 of $\geq 15\%$ was not achieved in all instances. Our patients were included on diagnostic criteria involving a positive conventional bronchial challenge; hence, we are not able to determine whether our allergen chamber exposure can elicit symptoms in asthmatic patients with a negative outcome of a conventional allergen bronchial challenge, but it seems unlikely.

Symptoms from other organs were noted by 10 patients. Symptoms from the eyes and nose dominated, but three patients registered itching from exposed skin areas in the active challenge. This illustrates the exposure chamber's ability to offer a "whole-body" allergenic load, able to induce symptoms in various organ systems. Horak et al. (4) found that, when using a constant load of fecal pellets from house-dust mites in the Vienna challenge chamber (VCC), symptoms occurred stepwise. First in the eyes, then in the nose, and, after a long time, in the lower airways of the patients. There is a marked difference between the mode of exposure used in the VCC and that used in our chamber. Both systems offer low "physiologic" allergen exposures, but the allergenic load is constant in the VCC, while we use an initial peak of nebulized allergen, followed by a clearing period of several hours (6). This fact might explain why we did not find a similar reaction pattern. In addition, Horak et al. have focused on patients with allergic rhinoconjunctivitis, while we have selected asthmatic subjects. The different populations of patients might respond differently.

By using standard components in the ventilation and sampling systems, we have been able to construct a functional exposure chamber at only moderate cost, when compared to the technically very sophisticated units described in other papers (1, 2, 9). The present trial illustrates the ability of our system to cause symptoms in the allergic subject, and not only in the lungs. We tried to establish a possible predictive factor for the outcome of allergen challenges, by comparing the results of the challenges with the allergen contents of dust samples obtained from the patients' mattresses. The patients with the highest allergenic load (≥ 2000 ng Der 1/g mattress dust) in their home environment tended to react faster than patients with low allergen levels. Theoretically, a "priming" effect could explain this observation; however, our patients were too few to confirm this. Our present study reflects some of the problems involved in all kinds of allergen challenge, due to the marked variation in individual responses to allergen exposure. Evaluating the symptom scores and medication of the patients was found to be

difficult due to the multiorgan involvement of house-dust-mite allergy. Because change in symptoms did not occur in parallel, no significant changes in medication or symptom scores could be seen in our relatively low number of patients.

For many years, the conventional bronchial challenge method with nebulizer and mouthpiece has been regarded as the "gold standard" for bronchial allergen exposures. However, from a physiologic viewpoint, the method is not convincing, and, not unexpectedly, the results differ from the results obtained in our exposure chamber, as the two methods apply very different amounts of allergen. The total dose is delivered within a few minutes in the conventional bronchial challenge, whereas the lower dose used in the exposure chamber is delivered to the controlled environment and then accumulates in the patient over a long time. Theoretically, the dose delivered in a conventional bronchial challenge, positive at 10000 SQ-U Der p, could be calculated as follows: 1.5 ml of each of all three allergen doses (100, 1000, and 10000 SQ-U/ml) was delivered to the patient, corresponding to a total dose of 1200 ng Der p. The mean concentration – if rapid and even distribution could be obtained – for the corresponding 3-h chamber challenge would be as follows: 1200 ng Der p/[12 m³ air in chamber+(3 h×4 m³ air delivered/h)]=50 ng Der p/m³ air. This allergen level corresponds to the findings of Price et al., who measured house-dust-mite allergen levels of 0–11.1 ng/m³ by high-volume air sampling, and ~60 ng/m³ by low-volume air sampling in the homes of asthmatic children (23). If the inspired air volume in 1 min is 6 l, a total allergen amount of the quantity (6 l/min × 180 min × 50 ng/1000 l air)=a total dose of 54 ng Der p administered to the patient during a full 3-h chamber challenge. Thus, a theoretic difference in total allergen dose of a factor of 10–20 is seen when comparing the different methods. Frølund found similar differences when comparing a single breath challenge method with a tidal breathing method for bronchial allergen challenge (7).

The symptoms observed in our trial subjects as a result of allergen exposures in the challenge chamber are expected to reflect the natural patterns seen in the average patient. The marked differences in the individual responses reflect the fluctuations in daily symptoms often observed in mite-allergic patients. These individual differences in sensitivity and patterns of reactions are often unobserved in a conventional bronchial challenge due to the unphysiologic and organ-targeted allergen exposure. Using an exposure chamber for allergen challenge could prove to be a way to validate bronchial hyperreactivity on a broader scale by using a method closer to the natural allergen exposure.

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