

Short communication

Effect of current exposure to Der p 1 on asthma symptoms, airway inflammation, and bronchial hyperresponsiveness in mite-allergic asthmatics

The existence of a dose-response relationship between indoor allergen exposure and sensitization has been widely described, but the effect of allergen exposure on asthma activity (symptoms, bronchial hyperresponsiveness [BHR], and inflammation) is not clear. Our aim was to determine the existence of an association among current exposure to mite allergens and symptoms, BHR, and airway inflammation assessed in blood and sputum from asthmatic patients sensitized to *Dermatophagoides pteronyssinus*. We selected 31 mild and recently diagnosed (12–24 months) asthma patients sensitized to *D. pteronyssinus*. Allergic exposure (Der p 1, Der 2) was assessed by a commercial assay based on monoclonal antibodies (mAb), carried out on the dust samples collected from patients' beds in a standardized way. Patients completed an asthma symptom questionnaire and underwent skin tests, methacholine bronchial challenge, and sputum induction. Sputum cell profile was analyzed and eosinophil cationic protein (ECP), tryptase, albumin, and interleukin(IL)-5 levels were quantified in sputum supernatant. Total eosinophil numbers and ECP levels were measured in blood samples. Most patients were exposed to Der p 1 levels under 2 µg/g of dust. Der p 1 exposure was higher among the subjects with positive sputum tryptase detection ($P=0.020$). Der p 1 levels showed a trend toward correlation with asthma symptoms ($P=0.066$, $r=0.36$) and correlated with sputum tryptase levels ($P=0.032$, $r=0.42$). No relationship between BHR, eosinophilic inflammation, and allergenic exposure was found. Our results suggest that asthma symptoms and lung mast-cell activation are at least partially dependent on current allergen exposure. The lack of correlation between mite exposure, eosinophilic inflammation, and BHR supports the role of other factors that enhance the immunologic response initiated by allergen, increasing the activity of asthma.

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There is extensive evidence that sensitization to perennial allergens plays a major role in the development of chronic asthma (1). Allergen bronchial provocation tests (A-BPT) have largely demonstrated that allergens can both induce and increase eosinophilic inflammation and bronchial hyperresponsiveness (BHR) (2). But data obtained with A-BPT cannot be compared with natural exposure: high doses of allergen are delivered in small particles and inhaled in short time periods in the A-BPT; meanwhile, small amounts of allergen airborne in large particles are inhaled during long periods of time in natural exposure (3). The measurement of mites collected from indoor reservoirs may differ from the allergen entering the lung, but most authors have failed to demonstrate significant amounts of airborne mite allergens in the absence of air disturbance (4). Therefore, to date, sampling of

reservoirs of home dust and the measurement of group 1 *Dermatophagoides* sp. allergens by a species-specific monoclonal antibody (mAb)-based ELISA is regarded as the most valid index of natural exposure (5).

Mite-avoidance experiments have demonstrated a significant decrease in asthma symptoms, airway eosinophilic inflammation, and BHR when the exposure of mite-allergic asthma patients to Der p 1 is decreased over 95% (6). But when a causal relationship between mite exposure and the severity of asthma symptoms has been investigated, the results have been controversial (7, 8). Such discrepancies can be attributed to many factors such as exposure to other allergens or to diesel particles (9), or respiratory viral infections (10) can act on the airways to upregulate their response to mite exposure. Secondly, several stimuli (exercise,

cold air, smoke) can trigger the “allergen-inflamed” airway causing acute asthma symptoms. Moreover, continuous exposure to indoor allergens can lead to chronic airway inflammation and to structural changes in bronchial architecture that increase asthma symptoms and BHR (11).

In this report, we studied a homogeneous group of mite-allergic mild and short-evolution asthma patients, and investigated the relationship between mite (Der p 1 and Der 2) exposure and the activity of asthma (clinical symptoms, BHR, and airway inflammation measured in blood and in induced sputum).

Material and methods

Subjects

We selected 31 asthma patients sensitized to *Dermatophagoides pteronyssinus* who attended our allergy service. The study was carried out from January to March 1996. The diagnosis of asthma was made according to international guidelines (12). Only short-acting β -agonists used “as needed” were allowed in the previous 2 months. A history of respiratory symptoms lasting 12–24 months was required. No patient had a history of smoking, had suffered from lower or upper respiratory tract infections during the previous 2 months, or had been treated with corticosteroids within the preceding 2 months. *D. pteronyssinus* sensitization was established by positive skin prick tests and specific IgE (Pharmacia CAP System, Pharmacia Diagnostics, Uppsala, Sweden) to this allergen in the context of a compatible clinical history. Patients sensitized to other perennial (animal dander, molds) or seasonal (pollens) allergens were excluded. All subjects were informed and gave their consent before starting the study. The ethics committee of our hospital approved the study protocol.

Study design

Patients underwent clinical and physical evaluation, and samples of venous blood and induced sputum were collected. Eight hours later, the methacholine bronchial provocation test (M-BPT) was carried out. A sample of dust from patients’ beds was collected. Patients completed a standardized asthma symptoms questionnaire of which the validity and reproducibility had been demonstrated (13). The frequency of presentation of 10 bronchial symptoms (dyspnea during exercise, diurnal resting dyspnea, diurnal wheezing, diurnal cough, nocturnal wheezing, nocturnal dyspnea, nocturnal cough, sleep disturbance, asthma anxiety, and chest tightness) during the last month was scored from 0 (never) to 4 (every day) points. The total symptoms score ranged from 0 to 40 points.

Cutaneous tests

Skin prick tests were carried out with a standard battery including the most common airborne allergens in our area (mites, pollens, molds, and animal dander). Tests were performed by the same technician, and the same lancets (ALK-Lancet, Abelló, Madrid, Spain) were used for puncture. The results of the skin tests were read at 15 min. Wheal diameters equal to or bigger than 3 mm were considered positive in the absence of response to phosphate-buffered saline (PBS). Histamine phosphate at 10 mg/ml and PBS were used as positive and negative controls, respectively.

We tested a *D. pteronyssinus* standardized extract (Abelló, Madrid, Spain), in which Der p 1 and Der 2 allergens had been quantified by mAb. The final extract at 100 biologic units (BU)/ml contained 40 μ g/ml of Der p 1 and 20 μ g/ml of Der 2. We skin-tested three concentrations of the extract (60, 12, and 2.4 μ g/ml of Der p 1) in duplicate and in inverse direction. The resulting wheals were shaped and transferred to millimeter-squared paper, where areas were measured by planimetry and expressed in mm². We considered for analysis the mean value of the skin areas obtained from the six tests.

Bronchial hyperresponsiveness (BHR)

To avoid circadian rhythm effect on airway dynamics, every BPT was carried out between 1400 and 1600. Nonspecific BHR was assessed with methacholine (Provocholine, Roche Laboratory, Nutley, NJ, USA) as agonist. Methacholine dilutions at 0.125, 0.25, 0.5, 1.0, 2.0, 5.0, 10.0, 25.0, 50.0, 100.0, and 200.0 mg/ml in PBS were made on the day of the test. Baseline spirometry was performed and forced expiratory volume in 1 s (FEV₁) was recorded. FEV₁ values equal to or higher than 70%-predicted normal were required to start the test. Patients had not inhaled β -agonist drugs for 8 h preceding the test. Diluent (PBS) and methacholine were administered by a Mefar dosimeter (Mefar s.r.l. Borezzo [BS], Italy), programmed to deliver five inhalations of 1 s each. Patients inhaled 10 μ l of solution in each inhalation. FEV₁ value was measured 3 min after inhalation, and a variability rate lower than 5% among basal and postdiluent FEV₁ values was required to start the test. Methacholine at increasing concentrations was administered by the dosimeter. The test finished when a fall in FEV₁ values equal to or higher than 20% from the postdiluent value was achieved, or when the highest concentration of methacholine was inhaled. Results were expressed in terms of the M-PD₂₀ (provocative cumulative dose of methacholine expressed in μ mol [1 mol methacholine=195.4 g] needed to decrease FEV₁ by 20% from the postdiluent value).

Sputum induction

Sputum induction was carried out 8 h before M-BPT in all the patients. Sputum samples were obtained by means of hypertonic saline inhalation according to Gershman et al. (14) with several modifications. The subjects inhaled four puffs of salbutamol 30 min before sputum induction. To avoid contamination of the sample, subjects were asked to rinse their mouth and clean their nose before induction, and when possible, before expectoration. An ultrasonic nebulizer (Ultraneb 99, De Vilbiss, Somerset, PA, USA) was used to administer saline at 5%, for three 10-min periods. After each period, the patients were asked to cough and expectorate into a sterile container; when possible, saliva was collected in a different recipient. The test finished when a macroscopically adequate sputum sample was obtained or when the three periods of inhalation were completed.

The volume of the entire sputum sample was measured, and 3 ml of the entire sputum sample was mixed with an equal volume of dithiotreitol (DTT) (Sputasol, Unipath Ltd, Basingstoke, Hampshire, UK) at 1/100, and rocked at room temperature for 15 min. Then, the mixture was put through one 0.42- μ m Millipore filter (Millipore, Somerset, PA, USA) and centrifuged at 1500 *g* for 10 min. The supernatant was then aliquoted and frozen at -70°C until further analysis. The pellet was suspended in saline serum at 0.9%, and totals of cells were counted with a Fuchs Rosenthal chamber. The suspension was then cytocentrifuged and stained with either Papanicolaou or Giemsa for differential cell counts. The sample was considered adequate for analysis when macrophages could be visualized and squamous cell contamination was lower than 20% (15). Percentage counts of macrophages, eosinophils, neutrophils, mast cells, and lymphocytes were made over a total count of 400 cells. A single person, blinded to the clinical conditions of the patients, performed the evaluation.

Soluble markers in sputum and serum

The concentrations of eosinophil cationic protein (ECP) and tryptase in the sputum supernatant, of serum ECP, and of serum total and specific IgE were measured with a fluoroenzyme immunosorbent assay (UniCAP, Pharmacia Diagnostics, Uppsala, Sweden). Sputum interleukin (IL)-5 levels were determined in duplicate by a commercial "sandwich" enzyme-linked immunosorbent assay (ELISA) (Quantikine; R&D Systems, Inc., Minneapolis, MN, USA). Sputum albumin levels were measured in duplicate by automated nephelometry (Behring Diagnostics GmbH, Marburg, Germany). The limit of detection for the fluid-phase assays was 2.0 $\mu\text{g/l}$ for ECP, 1.0 $\mu\text{g/l}$ for tryptase, 0.35 kU/l for specific IgE, 2.0 kU/l for total IgE, 3.0 $\mu\text{g/ml}$ for IL-5, and 1 mg/ml for albumin.

Sputum chemistry results were referred to the sputum and DTT mix volume. All assays were performed blind to the clinical details.

Collection of dust samples and quantitation of mite allergens

Dust collection was carried out 7 days after the last cleaning, as previously described (4). A portable vacuum cleaner provided with a prefilter (ALK, Denmark) which retains 74% of particles 0.3–0.5 μm in size, 81% of particles 0.5–1.0 μm , 95% of particles 1.0–10 μm , and 100% of larger particles (16) was used. The layer of bedding below the lower sheet, the layer above the upper sheet, and the area inside the pillow case were vacuumed for 2 min per m^2 . After vacuuming, the filter box was removed and stored in a plastic bag at -20°C until further analysis.

The content of major mite allergen (Der p 1 and Der 2) was determined in duplicate by a commercial ELISA with monoclonal antibodies (ALK, Denmark). Standard curves for these assays (1–200 ng allergen/ml) were routinely obtained from freeze-dried whole-mite extracts titrated against purified allergens. Allergen concentrations were estimated from three independent experiments. The mean intra-assay coefficient of variation was below 3%, while the interassay coefficient of variation from controls tested in all the assays was below 15%. The detection limit of the assays was 1 ng/ml.

Statistical analysis

Statistical analysis of the data was performed by the SPSS Windows 6.0 statistics program. Descriptive statistics were used to summarize the clinical and demographic characteristics of the patients. Results were expressed as median and interquartile range (IQR). PD_{20} values were log-transformed for analysis. Comparative analysis of groups was carried out by the Mann–Whitney U test. Correlation between variables was determined with the Spearman rank correlation coefficient. A *P* value of <0.05 was considered significant.

Results

We studied 31 asthma patients (55% male); the median age was 21 years (IQR: 19–29) and the median asthma score was 8 points (IQR: 5–12). The *D. pteronyssinus* median skin prick test area was 88.5 mm^2 (IQR: 72.2–109.0), and the median serum *D. pteronyssinus*-specific IgE was 44.9 kU/l (IQR: 19.2–58.5). The median value for serum total IgE was 174 kU/l (IQR: 110–292); for total blood eosinophil counts, 359 cell/mm^3 (IQR: 233–542); and for serum ECP, 16.8 $\mu\text{g/l}$ (IQR: 8.63–34.0). Median baseline FEV_1 values were 100% (IQR: 94–108); methacholine PD_{20} values were

achieved in every patient (individual values are displayed in Table 1). Sputum was safely induced in all the patients: median PEF decrease caused by the technique was 10.6% (IQR: 5.0–15.3%). Adequate sputum samples (alveolar macrophages visualized and squamous cell contamination under 20%) were obtained for every patient. The median value for sputum total cell counts was 768 cell/mm³ (IQR: 544–1762); for eosinophils, 10.0% (IQR: 3–20); for neutrophils, 22.0% (IQR: 10–34); for macrophages, 60.0% (IQR: 46–70); and for lymphocytes, 4.0% (IQR: 2–7). ECP and albumin were detected in most samples; tryptase was detected in eight (24%) of the sputum samples (Table 1), and IL-5 was undetectable in all the cases. Most patients (65%) were exposed to Der p 1 levels lower than 2 µg/g of dust (Table 1). Patients in whom sputum tryptase was detected had higher exposure to Der p 1 (median: 2.7 µg/g [IQR: 1.4–44.6] vs median: 0.7 µg/g [IQR: 0.2–2.5]) ($P=0.020$) and showed a trend toward higher Der 2 (median: 1.2 µg/g [IQR: 0.8–22.7] vs median: 0.4 µg/g [IQR: 0.1–1.7]) ($P=0.77$) levels. No differences in asthma symptoms, BHR, or sputum and blood-cell chemistry, were found

Table 1. Patients' individual data: asthma symptoms score, allergenic exposure, M-PD₂₀ values, and eosinophil numbers and chemical levels in sputum

Patient no.	Asthma score	M-PD ₂₀ (µmol)	Der p 1 (µg/g)	Der 2 (µg/g)	Sputum eosinophils (%)	Sputum ECP (µg/ml)	Sputum albumin (mg/ml)	Sputum tryptase (µg/ml)
1	9	0.20	NP	NP	26	15.2	0.1	1.5
2	5	10.0	1.43	0.81	16	37.7	52.4	3.5
3	6	1.90	2.35	1.24	49	122.0	82.9	81.6
4	11	1.20	44.62	22.72	4	21.5	61.0	4.61
5	26	3.60	2.67	0.77	2	849.0	119.0	13.1
6	3	3.60	59.24	37.31	10	16.9	10.16	2.0
7	11	0.40	26.74	11.86	51	63.6	62.7	2.99
8	3	47.90	0.51	0.17	1	12.1	40.4	2.22
9	12	5.20	2.41	1.37	17	49.2	0.0	0.0
10	4	4.0	0.28	0.06	3	19.0	0.0	0.0
11	16	10.27	NP	NP	2	6.07	9.61	0.0
12	8	63.10	0.1	0.1	1	14.0	10.8	0.0
13	11	190.0	7.06	1.85	0	11.3	0.0	0.0
14	8	9.30	0.40	0.16	11	13.0	7.21	0.0
15	15	190.0	1.28	0.30	29	69.6	24.4	0.0
16	13	1.60	1.23	0.33	7	10.4	32.0	0.0
17	7	4.80	0.64	1.03	20	43.0	4.83	0.0
18	8	1.00	2.5	0.53	6	3.2	0.0	0.0
19	11	15.61	0.12	5.23	8	18.4	18.7	0.0
20	19	1.70	NP	NP	20	27.0	5.7	0.0
21	5	4.80	0.15	0.07	31	13.9	0.0	0.0
22	4	0.43	0.21	0.21	10	7.09	9.37	0.0
23	7	1.90	3.30	8.95	7	45.6	17.4	0.0
24	13	20.90	1.84	0.91	10	77.6	32.5	0.0
25	6	3.60	0.05	0.85	36	103.0	13.5	0.0
26	19	7.60	0.85	0.27	23	75.6	39.1	0.0
27	2	190.0	NP	NP	14	10.1	9.56	0.0
28	3	1.76	0.23	0.08	2	13.5	19.3	0.0
29	2	4.40	0.09	0.08	20	503.0	13.3	0.0
30	12	0.30	21.3	7.93	1	3.18	7.94	0.0
31	8	30.20	12.27	3.45	4	13.1	8.84	0.0

M-PD₂₀: PD₂₀ methacholine; NP: not performed.

between the patients when they were grouped and compared according to their degree of Der p 1 exposure (equal to or higher than 2 µg/g and lower than 2 µg/g).

Sputum ECP levels correlated with eosinophil numbers ($P=0.002$, $r=0.54$) and albumin levels ($P=0.008$, $r=0.47$) and showed a trend toward correlation with tryptase ($P=0.093$, $r=0.57$) in sputum. Sputum tryptase and albumin levels were associated ($P=0.001$, $r=0.57$). Der p 1 levels exhibited a trend toward correlation with the score of symptoms ($P=0.066$, $r=0.36$) and correlated with sputum tryptase levels ($P=0.032$, $r=0.42$). A trend toward correlation between Der 2 levels and sputum tryptase ($P=0.092$, $r=0.33$) was also detected. No association among inflammatory indices, BHR, and allergen exposure was found.

Discussion

In the present report, we have evaluated the relationship between current exposure to major allergens of *D. pteronyssinus* and the activity of asthma in a homogeneous group of mite-allergic asthma patients. To exclude the possibility that sensitization to other allergens might interfere with the results, we studied *D. pteronyssinus*-mono-sensitized patients. To exclude the possibility that other airway inflammation enhancers (9, 10) might modify the asthma activity indices, we recruited nonsmoking patients who had not suffered from viral respiratory infections in the previous 2 months. To avoid the effect of the remodeling airway process on asthma symptoms, BHR, and airway inflammation (11), we studied a group of patients in whom asthma had been recently diagnosed (less than 2 years). According to other reports from our group (17) and from continental Europe (18, 19), most subjects were exposed to Der p 1 levels lower than 2 µg/g of dust.

We analyzed sputum samples for cells and for soluble markers of eosinophils and mast-cell activation (ECP and tryptase, respectively), of vascular leakage (albumin), and of eosinophilic chemotaxis (IL-5). According to other authors (14, 20), the detection of tryptase in sputum was low. Eosinophil numbers and ECP levels were lower than those reported in both entire (14, 15, 21) and selected sputum samples (20), and IL-5 was undetectable in all the samples. Our results might be imputed to the dilutional effect of saliva, but we used a "modified sputum process technique" in which saliva and sputum were collected in different containers, a method which significantly decreases saliva contamination (14). Thus, we are confident that our results were more likely to be due to the mildness of asthma than to the dilution of the sample. On the other hand, the high affinity of IL-5 for mucus might have induced its binding to mucus, interfering with its detection after filtering the sample (21).

Recent reports have described an association between indoor allergen exposure and asthma activity (22). In our study, Der p 1 exposure correlated with sputum tryptase levels, and a trend toward correlation between Der p 1 levels and asthma symptoms score was also found. Der p 1 exposure was higher among those asthma patients with detectable tryptase levels in sputum. Such a degree of relationship between mast-cell activation, asthma symptoms, and current allergenic exposure suggests that early allergen inhalation by sensitized asthma patients would activate bronchial mast cells that, through the release of preformed mediators, would elicit the acute asthma symptoms. The absence of a greater degree of relationship among such indices might be imputed to the low sputum tryptase detection or to the low exposure levels to Der p 1 presented by our patients. Other factors such as the highly variable perception of airway stenosis by asthmatics (23), or the effect that triggers (cold air, exercise, infections) can have on the inflamed airway determining asthma exacerbation (24) can lead to confusion when a dose-response relationship between exposure, mast-cell activity, and symptoms is tested.

Evidence that allergens both induce and enhance eosinophilic airway inflammation and BHR has been extensively provided by experimental trials from bronchial challenges (2) and from extreme allergen-avoidance studies (6, 25). Current indoor allergen exposure has also recently been related to BHR (26). In keeping with this, we would have expected to detect an association among allergenic exposure, eosinophilic inflammation, and BHR. We are aware that the lack of correlation found in our study might be due to the mildness of asthma or to the low allergenic exposure. However, on-going airway inflammation and BHR are

induced by repeated inhalation of low doses of allergen (27); therefore, it might be argued that neither condition is dependent on acute allergen exposure, but on the time and the allergen concentration that patients have been inhaling throughout their lives. Moreover, other factors should also be taken into account to explain the absence of a quantitative dose-response relationship among allergen exposure, eosinophilic inflammation, and BHR. Firstly, several agents (rhinovirus, endotoxins, diesel particles) have demonstrated their capacity to enhance the airway immunoinflammatory response initiated by allergens (9, 10). On the other hand, the genetic status of the patient results in a different degree of sensitivity and bronchial response to the allergen, and explains why the allergenic load does not differ between mite-sensitized asthmatic and nonasthmatic subjects (28).

In conclusion, current allergen inhalation is related to airway mast-cell activation and asthma symptoms. Airway eosinophilic inflammation and BHR might depend on the continuous allergen inhalation that leads to chronic inflammation. A longitudinal exposure trial in which the quantity of allergen that patients have been inhaling throughout their lives is known would have been a more accurate approximation with which to analyze the effect of perennial allergens in eosinophil bronchial inflammation and BHR.

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