

Repeated Inhalation of Low Doses of Cat Allergen That Do Not Induce Clinical Symptoms Increases Bronchial Hyperresponsiveness and Eosinophil Cationic Protein Levels

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Key Words

Eosinophil · Cat allergen · Inflammation · Bronchial hyperresponsiveness · Eosinophil cationic protein · Bronchial asthma

Abstract

The aim of this study was to investigate whether repeated exposure to subclinical doses of cat allergens, not inducing asthma symptoms, could affect eosinophil cationic protein (ECP) levels in bronchoalveolar lavage (BAL) or in peripheral blood, without the appearance of clinical symptoms. Twelve patients with mild asthma, all sensitized to cats and not exposed to cat allergen at home, underwent a series of inhalations of cat allergen or placebo for 8 days over 2 weeks. A methacholine challenge was performed before and after the allergen and saline exposures, and BAL and blood were sampled for ECP measurements and eosinophil counts. No patients experienced asthma symptoms. However, PD₂₀ methacholine (geometric mean) decreased significantly from 263 µg before to 126 µg after inhalation of allergen. Inhalation of saline did not induce any significant change in PD₂₀. The change in log PD₂₀ before and after cat allergen exposure was statistically different from the

change in log PD₂₀ before and after saline. Median ECP levels in BAL and serum increased significantly after allergen exposure, from 0.8 to 3.1 µg/l ($p < 0.02$) and from 15.9 to 31.4 µg/l ($p < 0.05$), respectively. No change was observed after saline inhalations. The change in BAL and serum ECP levels was statistically significant compared to that in the control group. The number of eosinophils did not change, however, nor did IL-5 and RANTES levels in BAL and serum. In conclusion, our results show that (1) exposure of asthma patients to repeated low doses of allergen, which did not provoke any clinical symptoms, is capable of inducing a local eosinophil activation associated with an increase in nonspecific bronchial hyperresponsiveness and (2) the increase in serum ECP levels due to eosinophil activation precedes the occurrence of asthma symptoms and may thus be a marker of allergen exposure in allergic asthma.

Introduction

Lung infiltration by inflammatory cells, especially eosinophils, is a characteristic pathologic feature of bronchial asthma [1] and has been associated with the increased

Table 1. Characteristics of study patients

Patients	Age years	Sex	Skin test to cat allergen	Specific IgE to cat allergen (class)	FEV ₁ before first methacholine (% predicted)	FEV ₁ before second methacholine (% predicted)	Maximal PEF variation ^a %	Dose of Fel d 1 BCT ^b ng	Daily dose of Fel d 1 exposure ^c ng	Airborne Fel d 1 at home ^d ng/m ³
1	36	F	+	3	78	74	10	38.5	7.0	<0.5
2	20	F	+	3	76	76	12	25.9	5.2	<0.5
3	24	F	+	4	103	98	2	332.5	70.0	<0.5
4	21	F	+	4	84	75	3.1	129.9	24.5	<0.5
5	21	F	+	3	88	79	3.6	11.9	2.4	<0.5
6	24	F	+	4	106	113	3	8.2	2.1	<0.5
7	23	M	+	3	93	94	1.7	61.2	12.2	<0.5

^a Maximum peak expiratory flow (PEF) variation: maximum difference in PEF, before and after inhalation of one low dose of cat allergen.

^b Cumulative dose of Fel d 1 inducing a positive conventional bronchial challenge test (BCT).

^c Daily dose of Fel d 1 to which patients were exposed during the low dose exposure challenge.

^d Airborne Fel d 1 measurements were done using a portable pump (Gilair) and a glass fiber filter during 8 h at 2 liters/min in the patients' homes.

bronchial hyperresponsiveness (BHR) observed in patients with asthma [2, 3]. Activated eosinophils may release their granular content, which includes eosinophil cationic protein (ECP) [4]. ECP has been detected in bronchial biopsies from patients with asthma and measured in their bronchoalveolar lavage (BAL) [5–10], sputum, and peripheral blood [11–13]. Serum and BAL ECP levels increase after natural exposure to allergens at symptom-inducing levels [14–16] and after positive bronchial [17–19] and nasal [20] allergen challenge tests.

BHR also occurs after a positive response to experimental bronchial allergen challenge tests [21] and after symptoms induced by natural exposure to allergen [22]. There are currently no data available, however, about the sequence of events involving BHR and inflammation before symptoms have occurred. Recently, Ihre and Zetterström [23] showed that exposure to low doses of allergen could induce an increase in BHR, but some of their subjects were, at times, symptomatic. We have developed a method by which asthma patients with cat allergies are administered repeated inhalations of low doses of cat allergen that induce no symptoms. We studied the changes in their BHR as well as in ECP levels in BAL and serum before and after allergen exposure.

Materials and Methods

Patients

This study included 12 patients with nonsymptomatic asthma who had been clinically sensitized to cat allergen and who gave written informed consent after a thorough explanation of the procedures, goals, and risks involved. Tables 1 and 2 report their baseline characteristics. Patients inhaled either cat allergen (table 1) or saline (table 2).

Inclusion criteria were: a history of cat-allergic asthma with a positive skin prick test to cat allergen extract (Stallergènes Pasteur, France), i.e. the mean diameter of the wheal obtained with the allergen extract was at least 75% of the mean diameter of the wheal induced by codeine 9%; specific IgE to cat allergen higher than class 2 (Matrix, Abbott Lab., Chicago, Ill.). All patients had forced expiratory volume in 1 s (FEV₁) greater than 70% of the predicted value and no clinical manifestations during the study period, a positive conventional bronchial challenge test to cat allergen performed 1 month before inclusion, and BHR to methacholine.

All the patients were free of medication for at least 1 month before the study period, except for the occasional use of inhaled β_2 -agonists. All were nonsmokers, and none had had a respiratory infection for at least 3 weeks before the study period.

Assessment of Domestic Allergen Exposure

None of the patients had a cat at home or any other contact with cats. To verify the absence of exposure to cat allergen at home, airborne Fel d 1 levels were measured twice, for 8 h each, with a glass fiber filter (AP 4003705, Millipore) connected to a portable pump at 2 liters/min, as previously described [24]. Fel d 1 was measured with a two-site monoclonal ELISA (Indoor Biotechnology, Chester, UK). The assay sensitivity was 0.5 ng/m³ at 2 liters/min during 8 h. The subjects were not exposed to any other allergens to which they were sensitized. In particular, the study was conducted out of the pollen season. Exposure to mites in mattress dust was assessed with the Acarex-Test® (Allergopharma, Hamburg, Germany).

Table 2. Characteristics of control patients

Patients	Age years	Sex	Skin test to cat allergen	Specific IgE to cat allergen (class)	FEV ₁ before first methacholine (% predicted)	FEV ₁ before second methacholine (% predicted)	Maximal PEF variation ^a %	Dose of Fel d 1 BCT ^b ng	Daily dose of saline ^c	Airborne Fel d 1 at home ^d ng/m ³
8	21	F	+	3	98	87	8	11.9	7	<0.5
9	24	F	+	4	116	115	6	8.2	6	<0.5
10	24	F	+	4	95	92	9.5	61.2	9	<0.5
11	29	F	+	4	80	102	12	0.7	6	<0.5
12	25	F	+	3	78	82	5	11.9	6	<0.5

^a Maximal peak expiratory flow (PEF) variation: maximum difference in PEF before and after one saline inhalation.

^b Cumulative dose of Fel d 1 inducing a positive conventional bronchial challenge test (BCT).

^c The number of inhalations was determined according to a theoretical number of nebulizations needed to inhale 1/5 of the Fel d 1 dose inducing a positive conventional bronchial challenge test.

^d Airborne Fel d 1 measurements were done using a portable pump (Gilair) and a glass fiber filter during 8 h at 2 liters/min in the patients' homes.

Study Design

Our study was a double-blind placebo-controlled study in parallel groups. It was approved by the ethics committee of our hospital. Subjects came on day 1 for a methacholine challenge test and blood sampling and on day 2 for bronchial fibroscopy. Then, for 5 consecutive days one week (days 5–9) and 3 consecutive days the following week (days 12–14), the subjects inhaled either low doses of cat allergen (1/5 of the dose that induced positive response) or saline. They returned on day 15 for a methacholine challenge test and on day 16 for bronchial fibroscopy and blood sampling.

Nonspecific BHR

A methacholine bronchial challenge test was performed. After baseline measurement of FEV₁ (Autospiro, Minato AS 500, Mediprom, Paris, France), the subject inhaled saline and then a 1% methacholine solution at concentrations in doubling increments from 25 to a maximum of 3,200 µg (cumulative). Inhalation took place through a mouthpiece, using a nebulizer (Mediprom FDC 88, Paris, France), and with the nose clipped. FEV₁ was measured 2 min after the saline inhalation and 2 min after each concentration of methacholine. The test stopped when FEV₁ had decreased by at least 20% from its postsaline value. The results were expressed as the challenge dose inducing a FEV₁ decrease of at least 20% after the dose-response curve to methacholine was constructed (PD20). After the onset of a positive response, the patient received a β₂-agonist inhalation and remained under observation for at least 1 h.

Allergen Inhalations

Conventional Cat Bronchial Challenge. The bronchial challenge was performed 1 month before the study began with an automatic inhalation-synchronized dosimeter jet nebulizer (Spira Elektro 2 inhalation dosimeter, Respiratory Care Center, Hameenlinna, Finland), as previously reported [25]. Briefly, each subject was instructed to breathe normally through the mouthpiece with the noseclip in place. At each dose level of cat allergen, the nebulizer delivered 7.1 µl of solution per breath, with nebulization time set at 0.5 s. FEV₁ was mea-

sured before and 15 min after the challenge. The nebulizer was filled with cat allergen solution, diluted to between 100 and 100,000 SQU/ml with the number of inhalations increasing progressively with the dose level (2, 4, and 8 breaths).

The starting concentration of cat allergen used was the skin end point obtained with the cat allergen extract. When the FEV₁ fell 20% or more within 15 min, the challenge was stopped. The cumulative total dose of cat allergen that induced a positive response was calculated. FEV₁ was then measured at 20, 30, 45 and 60 min, and every hour thereafter until it returned to its baseline value. After completing this challenge, subjects inhaled β₂-agonists and prednisolone as necessary. They remained under observation for at least 4 h after the last dose of antigen.

Inhalation of Low Doses of Allergen. On each of 8 days, each subject inhaled the same dose of cat allergen, that is one fifth of the dose that had induced a positive response. Control patients inhaled saline. Cat allergen extract (10⁶ SQU representing 350 ng of Fel d 1, Aquagen SQ, ALK, Denmark) or saline was administered by inhalation at the same time of the day (± 2 h) with a dosimeter (Spira Elektro 2). FEV₁ was recorded 5, 10, 15, 30 and 60 min after allergen or saline inhalation. The inhalation procedure was similar to that used for conventional challenge tests. Patients recorded their peak expiratory flow values twice daily throughout the study. Short-acting β₂-agonists were stopped at least 8 h before each test session.

Bronchial Fibroscopy and BAL

A fibroscopy was performed when FEV₁ was more than 70% of the predicted value. After premedication with diazepam (10 mg per os) and local anesthesia with 1% adrenaline-free xylocaine directly applied to the upper respiratory tract, a bronchial fibroscope (Olympus BF P30, Paris, France) was inserted through the nose into the trachea. Oximetry was recorded throughout the procedure. BAL was carried out in the middle lobe by instilling 4 times 50 ml of sterile isotonic saline gently aspirated immediately after instillation, as recommended by the NIH [26].

Patients received oxygen during bronchoscopy; an intravenous access was prepared, and adrenaline was readily available. Nebulization with salbutamol was performed before and after the fibroscopy. Oximetry monitoring continued for 4 h after the fibroscopy, and spirometry was performed every hour for 4 h.

ECP Measurements in BAL and Serum

The lavage fluid was immediately placed on ice and centrifuged at 4°C at 400 *g* for 10 min. The supernatant was lyophilized and stored in aliquots at -80°C until measurement of ECP in a 20× concentrated BAL. The serum was collected and allowed to rest for 1 h at room temperature (20°C) before it was centrifuged at 1,350 *g* for 10 min and stored at -80°C until ECP measurement. ECP levels below 2 µg/l were undetectable. ECP was measured in triplicate by a commercially available double-antibody radioimmunoassay (Pharmacia ECP RIA, Pharmacia Diagnostics, Saint-Quentin, France) [27, 28]. The intra-assay coefficient was 5.5% for 9.9 µg/l and 3.5% for 54.7 µg/l. The interassay coefficient was 12% for 9.9 µg/l and 17% for 54.7 µg/l. The recovery was 106±12% for the dilution tests and 91±3% for the addition tests.

Measurements of IL-5 and RANTES in BAL and Serum

IL-5 and RANTES were measured with a commercially available sandwich ELISA kit (Quantikine, R&D Systems, Oxon, UK). Briefly, 10× concentrated BAL or intact serum was added to microplates coated with a murine monoclonal antibody against human IL-5 or RANTES and then revealed with a polyclonal antibody against the same factors, conjugated to horseradish peroxidase. Next it was washed and revealed with the substrate (tetramethylbenzidine) and read at 450 nm with a correction at 540 nm in a microplate spectrofluorometer (Life Sci International, Cergy Pontoise, France). The limits of detection of IL-5 and RANTES were 3 and 5 pg/ml, respectively.

Eosinophils Counts in BAL and Serum

The cells from BAL were resuspended in Hanks' balanced salt solution without calcium and magnesium. The number of eosinophils in BAL was counted in 400 cells after cytocentrifugation (Cytospin®, Shandon Scientific, USA) processing 20,000 cells per slide stained by May-Grünwald-Giemsa. A differential blood count, with a Coulter Sysmex NE 9000 (Japan), determined the number of eosinophils in serum.

Expression of Results and Statistical Analysis

The cumulative dose of cat allergen inducing a 20% fall in FEV₁ was expressed in SQU or its Fel d 1 equivalent (10⁶ SQU represents 350 ng Fel d 1). After log transformation, PD20 were expressed as means ± SEM and geometric means in micrograms of methacholine (cumulative dose). Changes in log PD20 were analyzed by an unpaired *t* test. 95% confidence intervals were calculated for changes in log PD20 between before and after in each group, and between groups. ECP levels in BAL and serum were expressed as median, minimum and maximum, in µg/l in BAL and serum. RANTES levels were expressed as pg/ml in BAL fluid, and as ng/ml in serum. Percent eosinophil counts in BAL and serum were expressed as medians, minimum and maximum. Changes in ECP levels and eosinophil counts were compared between groups using a Mann-Whitney *U* test. The significance level was set at 5%.

Results

Patients

No patient experienced any asthmatic symptoms after the inhalation of cat allergen or after placebo. The mean variation of peak expiratory flow was 3.2% (range 0–12) after allergen and 3.5% (range 0–12) after saline (nonsignificant; tables 1, 2). FEV₁ did not vary by more than 5% in either group.

Domestic Allergen Exposure

No measurable airborne Fel d 1 was found in any of the patients' homes (<0.5 ng/m³; table 1). Moreover, the concentration of group 1 mite allergen was less than 2 µg/g in the mattress dust of mite-allergic patients, that is class 0 (Acarex-Test). The 3 patients sensitized to dogs did not have any dogs at home and had no contact with dogs throughout the experiment.

Nonspecific BHR

Baseline values were not significantly different between the two groups. Before inhalations began, PD20 was 263 µg methacholine. PD20 decreased significantly, to 126 µg, after the series of low-dose cat allergen inhalations (*p*<0.02) (fig. 1a, table 3). In contrast, saline inhalation did not induce any significant change in PD20, which was 108 µg methacholine before and 124 µg after exposure (fig. 1b, table 3). Moreover, the change in log PD20 before and after cat allergen was statistically different from the change in log PD20 before and after saline.

ECP Levels in BAL

Baseline values were similar between the two groups. Before cat allergen inhalation, ECP levels in BAL were 0.8±0.2 µg/l. They increased significantly, nearly quadrupling to 3.1±1.2 µg/l after exposure (*p*<0.02) (fig. 2a).

In contrast, inhalation of saline did not induce any significant change in BAL ECP levels (1.2±0.4 µg/l before and 1.3±0.2 µg/l after exposure) (fig. 2b). Changes in BAL ECP levels were significant between the groups (*p*<0.05).

Serum ECP Levels

Baseline values were similar between the two groups. Before cat allergen inhalation, serum ECP levels were 15.9±4.7 µg/l; after exposure, they doubled to 31.4±6.9 µg/l (*p*<0.05) (fig. 3a). In contrast, inhalation of saline did not induce changes in serum ECP levels (24.4±4.0 µg/l before and 27.9±5.1 µg/l after exposure) (fig. 3b). Changes in serum ECP levels were significant between the groups (*p*<0.05).

Table 3. Analysis of PD20 in the study and the control group

	Study	Control
log (PD20 before) ^a	5.5711±0.2748	4.6863±0.477
Geometric mean, µg methacholine	262.7	108.5
	NS	
log (PD20 after) ^a	4.8369±0.3667	4.8249±0.4387
Geometric mean, µg methacholine	126.1	124.6
	NS	
After – before ^a	–0.7342±0.2512	0.1386±0.274
95% CI	–1.3488; –0.1197	–0.6245; 0.9017
Geometric mean	0.4799	1.1487
	p = 0.02	
Difference of means ^a	–0.8729±0.3723	
95% CI for difference of means	–1.7152; –0.0306	

NS = Nonsignificant.
^a Mean ± SEM.

Fig. 1. Methacholine PD20 for individual patients obtained before and after inhalation of cat allergen (a) or saline (b). Geometric mean values are represented as gray blocks.

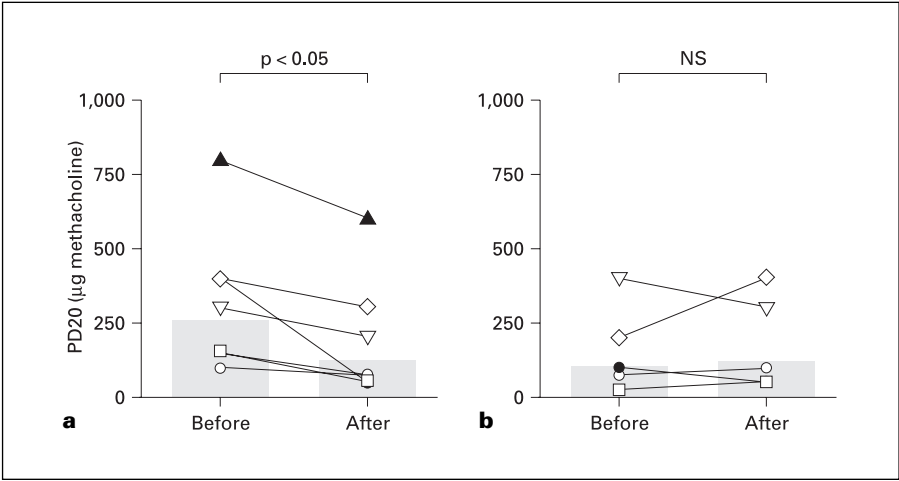


Fig. 2. Individual levels of ECP in BAL for patients before and after inhalation of cat allergen (a) or saline (b). Median values are represented as gray blocks.

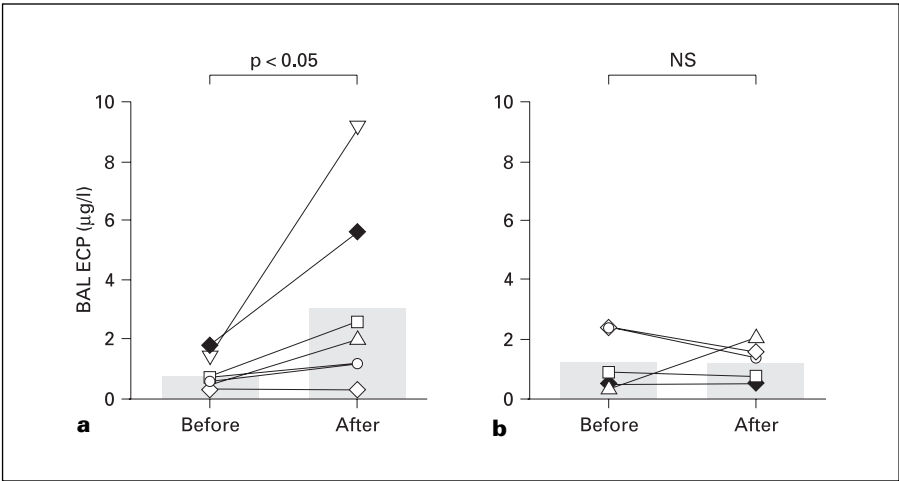
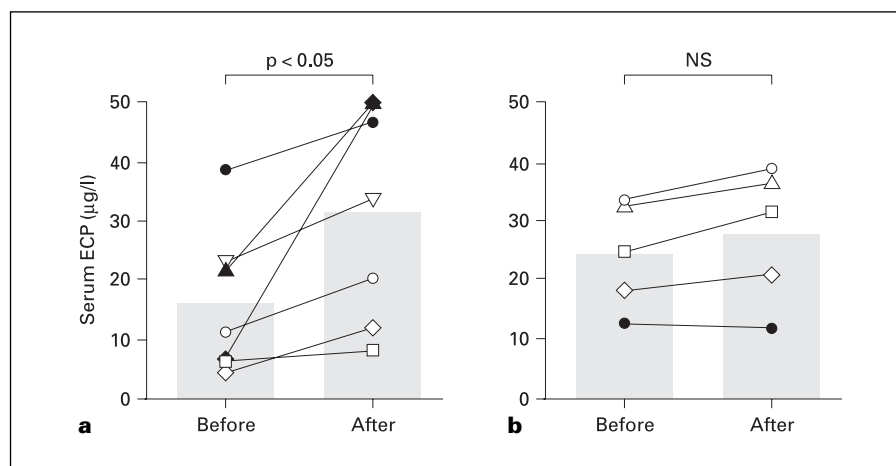


Fig. 3. Individual serum ECP levels for patients before and after inhalation of cat allergen (a) or saline (b). Median values are represented as gray blocks.



Eosinophil and Other Cells Counts in BAL and Serum

Baseline values were similar between the two groups. The number of eosinophils in BAL was similar before ($0.8 \pm 0.3 \times 10^3/l$) and after ($4.0 \pm 2.2 \times 10^3/l$) repeated inhalations of low doses of allergen, as well as before ($1.2 \pm 0.5 \times 10^3/l$) and after ($3.2 \pm 1.0 \times 10^3/l$) inhalations of saline. Similarly, the number of eosinophils in serum did not change significantly from before ($273 \pm 54 \times 10^6/l$) to after ($396 \pm 91 \times 10^6/l$) inhalations of low doses of allergen, or before ($310 \pm 87 \times 10^6/l$) and after ($340 \pm 90 \times 10^6/l$) inhalations of saline. No significant changes were found in the number of macrophages before and after challenge (4.5 and $8.7 \times 10^6/l$, respectively) and before and after saline (2.5 and $4.5 \times 10^6/l$, respectively). No significant difference was found in the number of lymphocytes, neither before and after cat allergen (0.27 and $0.42 \times 10^6/l$, respectively) nor before and after saline (0.3 and $0.2 \times 10^6/l$, respectively). The change in the number of macrophages or lymphocytes before and after cat allergen was not statistically different from the change in the number of macrophages or lymphocytes before and after saline.

IL-5 and RANTES in BAL and Serum

IL-5 levels in BAL and serum were nondetectable before and after allergen and saline inhalations. For RANTES, BAL levels did not differ significantly before (5.7 ± 2.1 pg/ml) and after (7.0 ± 4.3 pg/ml) allergen inhalations, or before (2.6 ± 1.0 pg/ml) and after (3.6 ± 1.5 pg/ml) saline inhalations; in serum, levels did not differ significantly before (29.3 ± 8.3 ng/ml) and after (29.4 ± 9.1 ng/ml) allergen, or before (27.7 ± 3.3) and after (34.5 ± 9.5 ng/ml) saline inhalations.

Discussion

The results of this placebo-controlled study show that inhalations of low, that is subclinical doses of allergens, in patients with mild asthma induced an increase in BHR associated with increased ECP levels in both BAL and serum, whereas inhalation of saline caused no changes.

One major strength of our study was the strict criteria for patient selection, based on both clinical history and experimental characterization of their cat-allergic asthma. All patients were free of symptoms at the time of the study. Moreover, the absence of measurable airborne Fel d 1 concentration in their domestic environment corroborated that they were not exposed to cat allergen. Our experimental protocol of inhalations of subclinical doses of cat allergen was based on several studies that showed that cat allergen doses that induced a positive response to a bronchial challenge test in experimental situations were significantly (5–60 times) greater than those provoking asthma symptoms at home [29, 30]. This suggested that exposure to lower doses of cat allergen might better reflect natural exposure to cat allergen and might thus help us understand the subclinical inflammatory changes that occur in the airways and prepare them for the asthma attack. We exposed asthmatic patients to doses one fifth as strong as those inducing symptoms in conventional bronchial challenge tests, so that we could monitor the changes that occur in the airways before asthma symptoms develop.

There is evidence that an increase in nonspecific BHR occurs in patients with asthma after a positive response to a conventional bronchial challenge test [21]. Recently, a pilot study among asthma patients sensitized to different allergens found an increased nonspecific BHR after repeated inhalations of subclinical allergen doses [23]. The patients

were, however, symptomatic at some points during the study, probably because the fixed doses of allergen were the same for all subjects (1 or 10 BU/ml). Indeed, the range of allergen doses that induces a significant response may widely vary from one subject to another [21, 31]. This is also true for cat allergen: the concentration of Fel d 1 inducing a positive response in bronchial challenge test is widely scattered [29, 30]. Our study provides confirmation: Fel d 1 levels inducing symptoms varied from 8.2 to 332.5 ng. When subjects received subclinical doses of cat allergen one fifth as strong as those that induced symptoms, they all exhibited increased BHR. The slope of the increase varied from one subject to another and was sometimes weak, as previously reported by Ihre and Zetterström [23]. The mean variation, however, was more than one doubling dose and is clinically significant [31]; the change in log PD₂₀ before and after cat allergen exposure was statistically different from the change in log PD₂₀ before and after saline. This finding corroborates that an increase in BHR may precede the occurrence of asthma symptoms in allergen-induced asthma. In another type of low-dose allergen challenge, Sulakvelidze et al. [32] found an increase in nonspecific hyperresponsiveness and airway eosinophils and IL-5. They also observed a significant increase in sputum of the percentage of EG2 cells and of ECP levels. However, some patients in this study experienced asthma symptoms.

The basal levels of ECP we found in BAL were similar to those reported by others for subjects with mild asthma [4, 6, 10, 33]. The ECP levels in BAL significantly increased, more than tripling, after repeated low-dose exposure to allergen. This increase is close to that observed after natural or experimental exposure to symptom-inducing allergen levels [19]. Although our study included only a limited number of patients, this more than 3-fold increase may be considered as the occurrence of local eosinophil activation in the airways induced by the low doses of cat allergen. It may indicate a progressive eosinophil activation in the airways before symptoms occur.

We also found a significant increase in serum ECP levels after inhalation of low doses of allergen. Increased serum ECP has already been reported for subjects with asthma after symptoms induced by each of the following: pollen season [14–16], alternate stays at high and low altitudes with subsequent natural allergen exposure [34], and symptomatic response to allergen bronchial challenge tests [12, 17]. The relationship between this eosinophil activation and BHR has been stressed by different authors in comparing results of symptomatic asthma patients and control subjects [14]. These results all suggest that serum ECP increases when an allergic patient becomes symptomatic. They do not

indicate, however, whether ECP might be a marker of exposure before symptoms develop in asthma patients. We have demonstrated here that ECP levels in serum may be directly related to the subject's allergen exposure. These results are in accordance with those of our prospective study of periodic asthma patients [15]: it shows that ECP is a marker of allergen exposure rather than of asthma severity.

These increases in the ECP levels of both BAL and serum were not associated with any increase in the number of eosinophils. One can suppose that the absence of a significant increase was a consequence of group size. Moreover, this finding could also indicate that the preexisting eosinophils, both locally in the airways and systemically in serum, were activated after inhalation of allergen. Others have contested the relationship between the increase in the number of eosinophils and airway hyperresponsiveness [35]. Our study provides evidence of a relationship between inflammation, in terms of eosinophil activation and BHR related to allergen exposure. This observation suggests that soluble factors, such as IL-5 or RANTES, may be secreted at the site of inflammation in the bronchi and may then contribute to peripheral activation of circulating eosinophils. Indeed, previous studies have shown such increases in IL-5 and RANTES between 4 and 8 h after challenge [36, 37]. However, we were unable to confirm any increase over baseline in IL-5 or RANTES levels in BAL or serum, 48 h after the last allergen inhalation. This result corroborates results by Fahy et al. [38] of a lack of increase in RANTES expression in the airways in asthmatics. Hence, if IL-5 or RANTES activate eosinophils after low-dose allergen challenge, this increase should occur earlier.

In conclusion, our results show that (1) exposure of asthma patients to repeated low doses of allergen, which did not provoke any clinical symptoms, is capable of inducing a local eosinophil activation associated with an increase in nonspecific BHR, (2) the increase in BHR and the release of ECP in the airways occur before asthma symptoms in allergic asthma, and (3) the increase in serum ECP levels, which is a consequence of the activation of eosinophils, precedes the occurrence of asthma symptoms and may thus appear as a marker of allergen exposure in allergic asthma.

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