

Brief communications

A simple method to evaluate basophil tryptase after allergen challenge in vitro

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Immunologic stimulation of basophils leads to increased gene expression and secretion of stored and newly synthesized mediators of the inflammatory process. Tryptase, the major neutral serine protease of human mast cells,¹ is absent from all peripheral blood leukocytes, with the exception of basophils where it is found at levels of approximately 0.4% of those in human lung mast cells.²

The small amounts of tryptase found in peripheral blood basophils are synthesized from small amounts of tryptase messenger RNA.³ Moreover, previous reports² showed that more tryptase was released from allergen-challenged basophils from patients with allergy and that nonspecific tryptase release was increased too.

Tryptase is released along with histamine by an IgE-mediated mechanism in vivo.⁴

The aim of this study was to investigate the use of a simple method to measure tryptase release from basophils after allergen challenge in vitro. For this purpose, we measured tryptase levels by sandwich radioimmunoassay in the supernatants of peripheral leukocyte suspensions.

METHODS

Subjects

Blood was taken from 298 patients with allergy and 170 nonallergic control subjects. Subjects underwent tests for allergy including medical history, skin prick tests, and specific IgE tests (CAP system; Pharmacia, Uppsala, Sweden).

Reagents

The incubation buffer was prepared as follows: piperazine-1,4-bis[2-ethanesulfonic acid], 3.02 gm/L (Merck); sodium acetate trihydrate ($\text{NaCH}_3\text{COO} \cdot 3\text{H}_2\text{O}$), 19.05 gm/L (Merck); potassium acetate (KCH_3COO), 0.49 gm/L (Merck); human serum albumin, 0.30 gm/L (Sigma); glucose ($\text{C}_6\text{H}_{12}\text{O}_6$), 1.00 gm/L (Mallinckrodt); calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), 0.147 gm/L (Merck).

The substances were dissolved in 900 ml of distilled water; the pH of the solution was adjusted to 7.4 with 1 mol/L Tris buffer, and the volume was adjusted to 1000 ml (the solution is stable for 1 week if stored at 2° to 8° C).

Allergens

The following liquid concentrated allergen extracts were available from Bülhmann Laboratories AG (Allschwil, Switzerland): grass mix, common birch, mites, cat epithelium, wall pellitory, olive, honeybee venom, and paper wasp venom.

Tests were performed within 3 hours of blood collection. Patients avoided use of corticosteroids, cromolyn, and antihistamines for 48 hours before blood sampling.

Seven milliliters of blood was collected into Vacutainer tubes (Becton Dickinson Vacutainer Systems) containing ethylenediaminetetraacetic acid and transferred into a 10 ml polypropylene tube containing 1.75 ml of 6% dextran solution. After an incubation for 90 minutes at room temperature, the upper phase was transferred into a second tube and centrifuged at 500 rpm for 15 minutes at room temperature. The cells were resuspended in 2 ml of buffer. Two hundred microliters of each cell suspension was pipetted into six small polypropylene tubes. At this step, 10 μl of cell suspension was mixed with 90 μl of 0-toluidine, and after incubation for 20 hours at +4° C, counted by Kova Glasstic Slide 10 with grids (Hycor Biomedical Inc.). Fifty microliters of buffer was pipetted into the first pair of tubes in which spontaneous release was evaluated; 50 μl of stimulation control (monoclonal antibody to IgE Le 27 at a concentration of 1 $\mu\text{g}/\text{ml}$) was pipetted into the second pair of tubes to obtain a positive control; 50 μg of allergen was added to the third pair of tubes.

After incubation for 40 minutes at 37° C, the tubes were centrifuged for 3 minutes at 3500 rpm, and 150 μl of cell supernatant from each tube were stored for up to 1 month at -20° C for radioimmunoassay procedures.

Tryptase

Tryptase levels of cell supernatants were determined by a sandwich radioimmunoassay as previously described⁵ by using iodine 125-labeled antibodies in coated tubes (Pharmacia).

The fluid was incubated in the tube, washed, incubated with a second antibody, and washed again; and the activity was measured in a gamma counter with standards for calibration. The lower limit of detection of the assay was 0.5 U/L (1 U = 1 μg of purified human lung tryptase).

Statistical analysis

Nonparametric statistical methods were used. The Mann-Whitney U test was used for comparison of two unpaired groups. Results were expressed as median values and were taken to be significant when *p* was less than 0.05. Correlation coefficients were determined by Spearman's rank correlation test.

Roc/Galen & Gambino analysis (Astute Statistic ADD-IN, 1993 DDU Software) was used to evaluate sensitivity, specificity, efficiency, and prevalence of the tryptase assay.

RESULTS

The amount of tryptase released from basophils of patients with allergy in response to Le 27 and allergen

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J Allergy Clin Immunol 1997;99:575-6.

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0091-6749/97 \$5.00 + 0 1/54/79716

TABLE I. Spontaneous tryptase release and in vitro tryptase release from basophils after stimulation with allergen extracts and monoclonal antibody anti-IgE (Le27)

	Nonallergic control subjects			Patients with allergy			<i>p</i> Value*
	Tryptase (U/L)			Tryptase (U/L)			
	<i>n</i>	Median	(2.5th-97.5th percentile)	<i>n</i>	Median	(2.5th-97.5th percentile)	
BCK	170	0.10	(0.00-2.40)	298	0.60	(0.00-3.10)	≤0.0069
Le27	170	2.40	(0.00-8.4)	298	2.90	(0.20-12.80)	≤0.0001
AE	150	1.40	(0.00-3.70)	298	2.10	(0.00-11.00)	≤0.0001

n, Number of subjects studied; BCK, spontaneous tryptase release; AE, allergen extracts.

*Determined by Mann-Whitney U test.

extracts was significantly higher than that released from basophils of nonallergic control subjects (Table I). Spontaneous tryptase release from basophils of patients with allergy was also significantly higher ($p = 0.0069$).

No correlation was found between tryptase levels and basophil number ($r = +0.24$, $p = \text{NS}$). Furthermore, no correlation was found between tryptase levels and serum IgE concentrations ($r = +0.4$, $p = \text{NS}$). On the basis of clinical findings (case history and skin prick test and CAP results), the numbers of positive and negative results of the tryptase release test were determined. The data were used for the calculation of sensitivity, specificity, prevalence, and overall efficiency (Roc/Galen & Gambino analysis). The results were as follows: 71.81%, 57.33%, 66.52%, and 66.96% (after allergen challenge); 71.81%, 35.88%, 63.68%, and 58.76% (after Le 27 challenge); 56.38%, 54.71%, 63.68%, and 55.77% (spontaneous tryptase release).

DISCUSSION

Basophils from patients with allergy released more tryptase than basophils from nonallergic control subjects, either spontaneously or after challenge with the relevant allergen in vitro. In addition, tryptase is considerably higher in the supernatant of basophils from patients with allergy after stimulation with anti-IgE Le 27. This may represent enhanced reactivity of basophils from patients with allergy.

Correlations with number of basophils and specific IgE appeared rather poor. This suggests that intrinsic cellular factors in addition to extrinsic factors, such as IgE, are important in determining basophil mediator release on challenge with activating signals.

The fact that the efficiency of the tryptase release test

was 66.96% after allergen challenge may be due to conditions that were technically not optimal. Moreover, the allergens used are not yet optimal, because the yield of positive results is too low. To improve the tryptase release test, it may be useful to use B12 monoclonal antibody, which recognizes both active and inactive tryptase.

Two main disadvantages of the tryptase release test can be identified: (1) false-positive results may be due to the action of some nonspecific cytotoxic agents, and (2) false-negative results may be due to hyporesponsive basophils or the low number of cells participating in the reaction. Because tryptase and histamine are both preformed mediators present in basophils, we could expect that their release kinetics are similar.

In this preliminary study, however, the histamine release was not determined in parallel with tryptase release. Nevertheless, the fact that no cells other than tryptase-containing basophils are involved in the reaction may be an advantage.

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Clothing is a carrier of cat allergens

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Clinical and allergologic studies have shown that sensitization to allergens derived from domestic animals (dogs and particularly cats) is an important cause of prolonged airway hyperresponsiveness with allergic respiratory symptoms and is sometimes a risk factor for bronchial asthma attacks leading to emergency department visits.¹

The major cat allergen, Fel d 1, is found universally in homes of patients with respiratory allergy induced by this animal. Significant increases in allergen levels have been detected after simply allowing the cats to leave their holding cages and move about the room. However, Fel d 1 is also present in dust samples from homes and public places where a cat has never been kept.² Consequently, not only direct exposure to cats but also indirect contact may be responsible for an allergic sensitization. In fact, many patients who are allergic to cats do not have this animal in their homes.

In this trial, to determine whether clothing could be responsible for dispersal of cat allergen, concentrations of Fel d 1 have been quantified in dust samples from clothes of subjects with and without a cat or a dog in their homes.

METHODS

Subjects

We collected dust directly from the winter clothes (skirts and trousers) of 33 subjects (12 with a cat, 11 with a dog, and 10 control subjects without these animals at home). We selected skirts and trousers because these clothes are more likely to have frequent contact with chairs and other surfaces on which cats and dogs may lay.

From each subject, two dust samples (each for 5 minutes and in two different parts of the same clothes) were collected. Clothes, sampled in situ, had not been washed or dry-cleaned in the last month.

High-volume sampling

For the dust collection from clothes, we used a high-volume vacuum air sampler (CF/20 Gelairé Flow Labs, Milan, Italy), which consists of a double-head membrane pump connected with two-flow control flowmeters and a volumetric counter. This device works with a constant airflow (20 L/min), and any

particulate material is harvested onto glass fiber filters with a diameter of 25 mm and a pore size of 2 μ m (borosilicate microfiber glass with acrylin resin binder, AP 20 Millipore; Vimodrome, Milan, Italy).

The filters have a measured efficiency of 84% for trapping all particles with a diameter more than 3 μ m. To reduce the risk of contamination, each filter was placed in a separate sealed tube. The device for the dust collection was also kept in a sealed box.

Assay of allergens in vacuumed dust samples

To each vacuumed dust sample, after vacuuming, was added (in Eppendorf tubes [Sigma Aldrich, Madrid, Spain]) 2 ml of phosphate-buffered saline with 0.2% bovine serum albumin. After centrifugation for 10 minutes at 10,000 rpm and filtration through a 0.45 μ m pore diameter membrane, the supernatants obtained were stored at -20° C until allergen assays were performed.

Fel d 1 content was determined by using a two-site ELISA. The method was a modification of previously described radioimmunoassay.³

Affinity-purified monoclonal antibody (5/24) was used at a concentration of 10 μ g/ml to coat a polystyrene plate. Standard curve (0.2 to 150 ng/ml) was made from a freeze-dried cat dander extract titrated against purified Fel d 1. Standard and samples diluted to the proper concentration were incubated for 2 hours. After washing, biotin-labeled secondary monoclonal antibody (C5/8) was added at a dilution of 1:1000. After incubation and washing, streptavidin peroxidase (Amersham) at a dilution of 1:5000 was added, and the enzymatic activity was evaluated by using o-phenylenediamine dihydrochloride as substrate.

Allergen concentration was estimated from duplicate absorbances of three different dilutions interpolated into the standard curve. Mean intraassay coefficient of variation was lower than 2%, and interassay variation determined with a control was lower than 7%. The lower quantification limit of the assay is 1.5 ng/ml (3 ng/filter).

Statistical analysis

The evaluation of statistical significance has been carried out by using a one-way variance analysis on log values of Fel d 1. This approach was necessary to normalize the variable distribution and to homogenize the variance. The analysis of variance was followed by Bonferroni's *t* test to detect significant differences among means.

RESULTS

All the samples, but two in particular, showed higher values than the quantification limits. Results of log of Fel d 1 allergen concentrations (expressed as nanograms per filter) in the three groups of patients are shown in Fig. 1. Statistical analysis of means appears to be significant ($F = 12.17$; $p < 0.005$).

Bonferroni's *t* test shows a statistically significant

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J Allergy Clin Immunol 1997;99:577-8.

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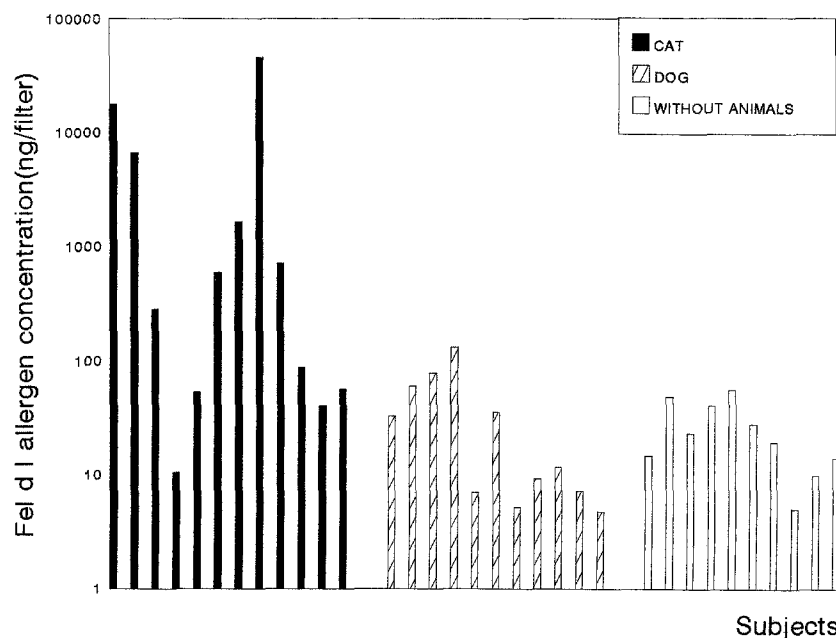


FIG. 1. Log of Fel d 1 allergen concentration (nanograms per filter) in clothes of subjects with a cat, subjects with a dog, and subjects without animals at home.

difference between the results for subjects with a cat versus subjects with a dog ($p < 0.05$) and subjects with a cat versus those without animals at home ($p < 0.05$). No statistical differences were found between the results of the group with a dog and those of the group without animals.

DISCUSSION

Several studies have shown that a significant proportion of airborne Fel d 1 is carried by particles of less than $2.5 \mu\text{m}$ in diameter, which can remain airborne for a long time even in undisturbed conditions.⁴ However, sometimes high concentrations of Fel d 1 have also been found in dust samples from homes where no cat has ever been kept. This allergen is very stable and ubiquitous, and its major source is direct shedding from cats themselves.²

Because one article has described the presence of mite allergens in clothes,⁵ we attempted to determine whether clothing is also responsible for the dispersion of cat allergen.

The concentrations of Fel d 1 were measured directly in dust samples taken from the clothes (skirts and trousers) of subjects with and without cats in their homes. Because we excluded the possibility that Fel d 1 could contaminate the filters and the sampling instrument, the only source of this allergen in our trial was the clothing. This hypothesis is also supported by our pre-

liminary observation of a limited number of subjects, who showed a dramatic reduction in the amount of Fel d 1 in clothing after washing. As a consequence, clothing may constitute an important means for the distribution of this allergen in cat-free environments and may consequently also represent a risk factor for triggering asthma in cat-sensitized individuals.

As a result of this study, we speculate that to reduce the risk of cat allergy, it is important to wash frequently not only the cat but also the clothing of persons with a cat at home. Further studies of this subject are currently in progress in our laboratories.

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