

Dog Serum Albumin as an Allergen

IgE, IgG and Lymphocyte Responses in Dog Dander-Sensitive Asthmatic Children

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Abstract. To study the allergenicity of dog serum albumin (DSA) in dog-sensitive subjects, we investigated 203 asthmatic children by means of the skin prick test (SPT), the radioallergosorbent test (RAST) and the in vitro lymphocyte stimulation test. Significant SPT reactions to DSA were observed in only 9 out of 80 subjects with a significantly positive SPT reaction to dog dander and hair (DDH). Analogously, positive DSA RAST results were observed only in a minority of the cases with a positive DDH RAST. Significant SPT reactions to DSA were not observed in subjects with a negative clinical history of dog allergy, or in subjects with a negative reaction to DDH in the SPT or in the provocation test. DSA induced remarkable lymphocyte stimulation in only 1 subject, who also had a significant SPT reaction to DSA. DSA-specific serum IgG antibody titers measured by ELISA were not found to correlate with DSA SPT or DSA RAST.

Introduction

Animal dander constitutes an important group of inhalant allergens. Among domestic animals, cat and dog are the most common sensitizers in different parts of the world [5, 7, 13, 20]. Several studies concerning animal dander allergens have indicated the possible importance of serum albumin as an allergen in dander extracts. The first investigation to demonstrate it was carried out as early as 1942-43 by *Squire* [14], who in his profound study found evidence of albumin being an important allergen in horse dandruff. This was confirmed later by more advanced immunological techniques in a study by *Ponterius* et al. [12]. *Lowenstein* et al. [9] classified serum albumin as a minor allergen in horse hair and dandruff. *Holford-Strevens* [6] investigated cat skin extract and found that the most active fraction of it migrated with an electrophoretic mobility similar to that of serum albumin. *Stokes and Turner* [15] analyzed cat allergens and found that serum albumin was one of the two most important allergens. They, however, demonstrated that serum albumin made a quantitatively minor contribution to the total allergenic activity of cat extract. This was confirmed by *Ohman* et al. [11], who showed that only a minority of cat-sensitive patients had IgE antibody to cat serum components.

Dog dandruff has been studied analogously. *Yman* et al. [19] investigated allergens from dog serum and extracts of dog hair, dandruff and skin scrapings by polyacrylamide gel electrophoresis, immunodiffusion and radioimmunoassay, and found evidence of dog serum albumin (DSA) as an important allergen in dog epithelia extracts. This was confirmed by *Blands* et al. [2] using crossed radioimmunoelectrophoresis; DSA was suggested to be an important major allergen in dog dander. However, *Brandt and Yman* [3] could find IgE antibodies to dog serum in only 4 out of 38 patients with hypersensitivity to dog. *McLean* et al. [10] found positive skin test reactions to DSA in 4 out of 10 patients with histories of dog hypersensitivity; dog albumin-specific IgE was observed in 5 out of 9 patients.

Despite the slightly controversial data the importance of serum albumin as an allergen, no extensive in vivo allergy tests with serum albumin has been reported. The purpose of the present study was to investigate the importance of DSA as an allergen in dog-sensitive asthmatic children. The IgE responses to DSA were measured by routine allergy tests, the skin prick test (SPT) and the radioallergosorbent test (RAST). The DSA specific IgG antibodies were measured by the ELISA technique. In vitro lymphocyte transformation by DSA was studied in a part of the subjects.

Material and Methods

Patients

203 children from the Children's Asthma Register of the Pediatric Department of the University of Turku [8] were included in the study. The age of the children ranged from 4 to 15 years; 60% of the subjects were males. None of them had previously been hyposensitized with dog or cat allergen extracts. The clinical history of animal dander allergy was obtained by means of a detailed questionnaire, and the subjects were divided into three groups according to the clinical history of dog allergy: (a) negative, (b) doubtful, and (c) positive.

Allergen Extracts

Dog dander and hair (DDH) extracts were obtained from the Allergologisk Laboratorium A/S (Copenhagen, Denmark). The raw material for the extract has, according to the manufacturer, been collected from different dog breeds. The same batch (Hu 073) of the purified, freeze-dried extract was used throughout the study. The concentration of the DDH extract in the SPT was 1 mg/ml, corresponding approximately to 1:20 w/v; the allergenic activity of the extract according to the manufacturer and our own studies [16] was approximately 1 HEP.

DSA was kindly supplied by the Allergologisk Laboratorium A/S. The purity of the albumin was tested by immunoelectrophoresis using antisera against (a) dog albumin and (b) dog serum proteins (Nordic Immunological Laboratories, Tilburg, The Netherlands). The concentration of DSA solution in the SPT was 0.25 mg/ml.

Skin Prick Tests

The method recommended by the Northern Society for Allergy [1] was followed. In each patient a test with the negative control solution (the diluent provided by the manufacturer) was performed. A wheal size equal to the reaction of the positive reference (histamine hydrochloride 1 mg/ml) was recorded as a +++ reaction. The skin reactions were measured after 15–20 min.

Provocation Tests

Dog allergy was confirmed by the provocation test with the aqueous DDH extract. Either the bronchial and/or the conjunctival provocation test was used, as described elsewhere [16].

RAST Analyses

RAST analyses were performed with DSA and with DDH coupled to activated filter paper discs according to Ceska et al. [4], as described elsewhere [17]. The coupling was performed in an allergen concentration of 2 mg/ml. The results were expressed as a percentage of the total activity.

IgG Determinations

IgG antibodies to DSA and DDH were measured by the enzyme-linked immunosorbent assay (ELISA) [18]. ELISA was measured in special polystyrene microtiter plates (Linbro Division, Flow Lab. Inc., Hamden, Conn.) with 96 wells constructed for a semiautomatic analyzer (Titertek Multiskan, Eflab, Helsinki, Finland; product of Flow Lab. Inc.). Each well of the plate was coated with 120 μ l of (a) DSA 1 μ g/ml, by incubation at 37°C overnight. After incubation, the plates were washed with PBS and then saturated by 2 h incubation with 100 μ l of PBS containing 1% normal

sheep serum (NSS-PBS) in order to reduce nonspecific absorption of proteins onto the solid phase.

The patient sera were diluted in 1:50 in NSS-PBS, and 75 μ l of this dilution was added to the antigen-coated wells and incubated at 37°C for 2 h. After washing three times with 0.9% NaCl containing 0.05% Tween 20 (NaCl-Tw), 75 μ l of 1:200 dilution (in 5% PBS-NSS) of alkaline phosphatase conjugated swine antibodies against human IgG (Orion Diagnostica, Espoo, Finland) were added. After incubation overnight at room temperature the plates were again washed three times with NaCl-Tw. The amount of alkaline phosphatase bound to the plates was quantified using *p*-nitrophenylphosphate (NPP, Sigma Chemical, Co., St. Louis, Mo.) as a substrate in glycine-Mg-Cl₂ buffer, pH 10 (Orion Diagnostica). The optical absorbance was measured at a wavelength of 405 nm by Titertek Multiskan. A standard curve was prepared daily for each assay using a highly positive reference serum as a standard. The same reference serum was used throughout the study. The concentration of the IgG antibodies in the sample was expressed as a percentage of the antibody concentration in the reference serum.

In vitro Lymphocyte Transformation

Lymphocyte stimulation by DSA and by DDH extracts was done in whole blood microcultures [18] with slight modifications. In brief, 2 ml of heparinized blood was mixed with 10 ml of RPMI (Grand Island Biological Co., Grand Island, N.Y.) containing antibiotics. Thereafter, 150 μ l of the blood-RPMI suspension was distributed to each well of a microtiter plate (IS-MRC-96, Linbro Chemical, New Haven, Conn.). Different dilutions of the allergen extract (DSA 5, 50 and 500 mg/ml) of PPD (Statens Seruminstitut, Copenhagen, Denmark) and streptokinase-streptodornase (SK-SD; Varidase, Oy Drugtrade Ab, Helsinki, Finland; a product of Lederle Laboratories, Madrid, Spain) in 25 μ l of RPMI were added to each well. Control cultures received 25 μ l of the plain medium. All cultures were prepared in triplicate. The plates were incubated for 96 h in humidified air and 5% CO₂ at 37°C.

To measure the degree of lymphocyte transformation 200 μ l (0.25 μ Ci) of [¹²⁵I]-5-iodo-2'-deoxyuridine (spec. act. 90–110 mCi/mg; Radiochemical Centre, Amersham, England) was added into each well together with 5-fluoro-2'-deoxyuridine (Fluka, Buchs, Switzerland) to a final concentration of 10⁻⁶ M. 18 h later the cultures were harvested with a multiple cell-culture harvester (Skatron, Lierbyen, Norway) and the radioactivity was measured by a gamma counter. The responses are expressed as net counts (cpms of the stimulated culture minus cpms of the control culture).

Statistical Methods

The statistical significance of the differences between the two means was tested by using the Student's *t* test after having applied the *F* test. The statistical significance of the differences between the observed group distributions was tested by using the χ^2 test. In tables I–IV, Yates' correction for continuity was used. The correlation coefficients were calculated with the aid of Pearson's formula for the product-moment correlation coefficient.

Results

In the immunoelectrophoresis the DSA solution was found to be pure; a single precipitating line was

Table I. SPT results with DSA compared with clinical history of dog allergy (χ^2 12.06, d.f. 1, $p < 0.001$)

SPT with DSA	Clinical history of dog allergy			
	negative	doubtful	positive	total
0	67	43	54	164
+ or ++	4	5	11	20
≥ +++	0	0	9	9
*total	71	48	74	193

Table II. SPT results with DSA compared with provocation test reaction to DDH (χ^2 7.02, d.f. 1, $p < 0.01$)

SPT with DSA	Provocation test result with DDH			
	negative	doubtful	positive	total
0	96	7	61	164
+ or ++	9	0	11	20
≥ +++	0	1	8	9
*total	105	8	80	193

Table III. SPT reactions to DSA compared with reactions to DDH (χ^2 9.42, d.f. 1, $p < 0.01$)

SPT result with DSA	SPT result with DDH			
	0	+ or ++	≥ +++	total
0	55	48	61	164
+ or ++	1	9	10	20
≥ +++	0	0	9	9
*total	56	57	80	193

Table IV. SPT reactions to DSA compared with DSA RAST

DSA RAST, cpm%	SPT result with DSA		
	0	+ or ++	≥ +++
Mean	0.077	0.130**	1.384*
SD	0.044	0.211	0.806
n	159	19	9

* $p < 0.001$ when compared to the two other groups (*t* test).

** $p < 0.01$ when compared to those with negative DSA SPT.

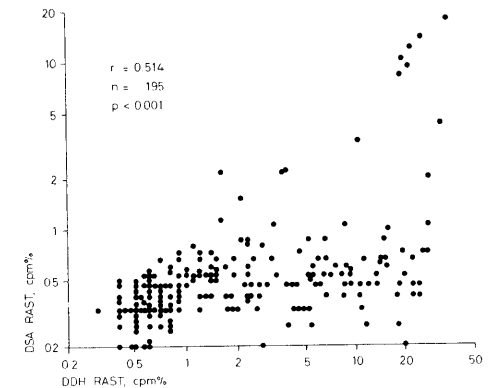


Fig. 1. Correlation between DSA RAST and DDH RAST.

observed both with antisera against dog albumin and antisera against dog serum proteins.

The SPT with DSA gave significant reactions (≥ +++) in 9 out of the 74 subjects with a positive clinical history of dog allergy (table I). Significant skin test reactions to DSA were not observed in 71 subjects with a negative clinical history of dog allergy; however, in 4 of them a slight (+ or ++) SPT reaction was elicited. 2 out of these 4 cases had a positive clinical history of cat allergy.

A significant DSA SPT reaction was observed in 8 out of 70 cases with a positive provocation test result by DDH extract (table II). Slight SPT reactions to DSA were observed in an additional 11 cases. Among the cases with a negative provocation test no significant SPT reactions to DSA were observed; slight reactions were elicited in 9 out of 108 cases with a negative provocation test result. Similar results were observed when SPT results with DSA were compared with SPT results with DDH extract: a positive skin reaction to DSA was observed in only 28 out of 137 cases with a positive reaction to DDH (table III).

The RAST results were consistent with the SPT results (fig. 1): strongly positive (≥ 5 cpm%) results in DSA RAST were observed in only a minority of the subjects with a strongly positive DDH RAST. In cases with a negative DDH RAST the DSA RAST was also negative. The DSA RAST results were in good agreement with DSA SPT (table IV) which also suggests that the test results to DSA were not unspecific.

The DAS-specific IgG antibody titer correlated positively with the DDH-specific IgG antibody titer ($r = 0.500$, $n = 198$, $p < 0.001$), but neither with DDH- nor DSA-specific IgE antibody titers ($r = 0.070$ and 0.120 , respectively, $n = 195$). DSA induced a remarkable lymphocyte transformation in only 1 subject, who also had a strongly positive DSA skin reaction.

Discussion

Our results show that DSA is not a major allergen in DDH; rather, it is a minor or an intermediate allergen. According to the tests used in our study, most of the dog-allergic asthmatic children showed no indications of allergy to DSA. Clearly positive signs of DSA allergy were not observed in subjects without dog allergy, which suggests that the test reactions to DSA were not unspecific. Also the good agreement between DSA RAST and DSA SPT gives evidence of this.

In our study, DSA-specific IgG antibody titers were found to correlate with DDH-specific IgG antibodies, but the concentration of these specific IgG antibodies in sera did not correlate with the DSA or DDH allergy. No evidence of the 'blocking' or 'sensitizing' character of serum IgG antibodies could be shown. However, the various IgG subclasses were not analyzed in our study.

DSA was shown to be a weak stimulator of the in vitro lymphocyte transformation in the concentrations used in our study. It is possible, though not probable, that an effect of DSA on the in vitro lymphocyte transformation could have been shown had a wider scale of antigen concentration been used. However, our unpublished data have shown that even DDH could not induce a significant in vitro lymphocyte transformation.

In some previous studies [2, 10, 19] DSA has been regarded as an important allergen in DDH, even as a major allergen. An explanation for this could be the difference in the populations of the different studies. If the subjects for the studies were selected on the basis of the positive SPT reaction to a poorly standardized DDH preparation, which probably contained great amounts of dog serum proteins and less dandruff allergens, biased results could probably have been obtained. In our study, the subjects for the study were not selected on the basis of dog allergy tests, but all subjects of certain age groups from the Children's

Asthma Register were investigated, using standardized [16] allergen extracts and test methods. The results of our study are in good agreement with those of Brandt and Yman [3], who found IgE antibodies to dog serum proteins only in a minority of the subjects with dog allergy. In our opinion, DSA can thus be regarded as a minor allergen in dog-sensitive subjects.

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