

False-positive skin prick test responses to commercially available dog dander extracts caused by contamination with house dust mite (*Dermatophagoides pteronyssinus*) allergens

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Background: In an outpatient population, a high frequency of positive skin prick test responses to dog dander was found in the absence of detectable IgE to dog dander in the RAST. The majority of these patients were sensitized to house dust mites (*Dermatophagoides pteronyssinus*) and had no obvious dog-related allergic symptoms. These findings prompted us to investigate whether dog dander skin test preparations are contaminated with house dust mite allergens in amounts sufficient to cause false-positive skin prick test responses in patients sensitized to house dust mites.

Methods: Antigen detection assays with monoclonal and polyclonal antibodies were used to determine concentrations of the major allergen Can f 1 from dog dander and the major allergens Der p 1 and Der p 2 from house dust mites in five commercially available dog dander skin prick test preparations (A to E).

Results: Can f 1 concentrations varied for the different extracts (A: 170 µg/ml, B: 11.1 µg/ml, C: 13.3 µg/ml, D: 3.8 µg/ml, and E: 59.4 µg/ml). Der p 1 was detectable in all extracts (A: 33.4 ng/ml, B: 5.1 ng/ml, C: 29.6 ng/ml, D: 0.4 ng/ml, and E: 1.9 ng/ml), and Der p 2 was detectable in some of the commercially available dog dander skin prick test preparations tested (A: 31.3 ng/ml, B: 3.0 ng/ml, and C: 7.5 ng/ml). The median house dust mite threshold in the skin prick test was found to be 5.8 ng/ml of Der p 1 (range, 3.5 to 20.8 ng/ml) in nine patients tested.

Conclusion: Contamination of commercially available dog dander skin prick test preparations with the major allergens (Der p 1 and Der p 2) of the house dust mite (*D. pteronyssinus*) was demonstrated. These contaminations cause false-positive responses to skin prick tests with dog dander in patients sensitized to house dust mite. (*J Allergy Clin Immunol* 1996;98:1028-34.)

Key words: Skin prick test, dog dander, *Dermatophagoides pteronyssinus*, RAST

In our outpatient department we routinely test new patients who display respiratory symptoms with the skin prick test (SPT) and the RAST. A

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Abbreviations used

CNBr:	Cyanogen bromide
HDM:	House dust mite
SPT:	Skin prick test

series of aeroallergens common in The Netherlands are tested, including house dust mite (HDM) (*Dermatophagoides pteronyssinus*) and dog dander. In patients sensitized to HDM, as indicated by a positive HDM SPT response and RAST score, we frequently find positive responses to SPTs with dog dander in the absence of detectable levels of

anti-dog dander IgE in the RAST. In addition, dog-related allergic symptoms are not common in these patients.

IgE to minor components or contaminants is more easily detected in the skin test than in the RAST.¹ A possible explanation of our finding of discrepancies between dog dander SPT and dog dander RAST results could be contamination of the dog dander SPT extract with HDM allergens, causing false-positive dog dander SPT responses in patients highly sensitized to HDM.

Contamination of allergen extracts for skin tests with HDM allergens was previously found in cat dander extract² and has been suggested for hen's feather extract.³ We therefore analyzed five commercially available dog dander SPT preparations for contamination with the HDM major allergens Der p 1 and Der p 2 using antigen detection assays with monoclonal and polyclonal antibodies. In addition, the minimum concentration of a HDM extract that will cause a positive SPT response was determined in a group of patients highly sensitized to HDM.

METHODS

Patients

SPTs and RASTs were performed routinely in 1299 patients with respiratory complaints who visited the outpatient clinic of the Academic Medical Center between May 1993 and July 1995. In the SPT and RAST, a series of important aeroallergens in the Netherlands were tested (*D. pteronyssinus*; cat, dog, guinea pig, and rabbit hair or dander; mugwort, grass [mixture], and tree [mixture] pollen; *Alternaria alternata*; *Aspergillus fumigatus*; and *Cladosporium herbarum*). By using a standard questionnaire concerning allergy-related symptoms, a history of allergy was documented in 1192 (92%) of these patients during a short interview.

A subgroup of 10 patients (patients I to X) with positive responses to dog dander SPT and no detectable anti-dog dander IgE, and a high level (RAST class 4+ or 5+) of anti-HDM IgE was selected for further serologic evaluation (see RAST).

SPTs

The SPTs were performed and documented by two investigators (M. J. V. and A. M. W.) according to a standardized procedure.⁴ A positive test response was defined as a skin reaction with a wheal greater than 3 mm in diameter or at least half the diameter of a 10 mg/ml histamine-HCl SPT, surrounded by erythema, and occurring in combination with a negative response to SPT with the control fluid (phosphate buffered saline, 50% glycerol, 0.5% phenol).

The Der p 1 content of the threshold concentration of an HDM extract causing a positive SPT response, was

assessed for nine patients with HDM RAST scores of 4+ or 5+. Duplicates of twofold dilutions of an HDM SPT extract with known Der p 1 content were tested until a reaction with a wheal diameter less than 3 mm was obtained. The concentration of Der p 1 was calculated for the dilution, found by interpolation, that resulted in a wheal diameter of 3 mm.

To investigate whether variations in contamination of dog dander SPT extracts by HDM allergens resulted in gradations of skin test reactivity, we performed SPTs with five dog dander SPT preparations, A to E, in four patients with HDM RAST scores of 4+ or 5+, no history of allergy to dogs, and no detectable anti-dog dander IgE in the RAST (patients 1 to 4). The SPTs were performed in duplicate, and the results are expressed as the mean of the maximal and orthogonal diameter of the wheal and flare skin reaction.

RAST and RAST inhibition

For the routine detection of IgE antibodies to dog dander, we used an indirect RAST system with hapten-modified allergens as described previously.⁵ In this dog dander RAST, with dog dander extract F (see Table I), approximately 34.2 ng Can f 1, 6.5 pg Der p 1, and 4.6 pg Der p 2 was bound per test to the solid phase. For the HDM (*D. pteronyssinus*) RAST, the allergens were insolubilized by coupling to cyanogen bromide (CNBr)-activated Sepharose (Pharmacia, Uppsala, Sweden)⁶ resulting in approximately 750 ng Der p 1 and 840 ng Der p 2 binding per test. In each test, a 50 µl sample of patient serum was incubated overnight. After washing, iodine 125-labeled sheep anti-human IgE was added and incubated overnight. The percentage of radioactivity bound was assessed after a wash step. The RAST results were first corrected for nonspecific binding (less than 1% binding of radioactivity), and scores were assigned as follows: 0—less than 2% binding; 1+—2% to 5% binding; 2+—5% to 10% binding; 3+—10% to 20% binding; 4+—20% to 40% binding; and 5+—greater than 40% binding.

The sera of patients I to X were retested in a modified RAST with a concentrated dog dander extract, originating from an identical preparation as used in the SPT (dog dander extract G; see Table I), bound to CNBr-activated Sepharose in high concentration (20 µg protein, 4.1 µg Can f 1, 0.9 ng Der p 1, and 1.1 ng Der p 2 per test).

For RAST inhibition of this concentrated dog RAST in patients I to X, serum samples were preincubated for 2 hours with 50 µl of a *D. pteronyssinus* whole body extract (Commonwealth Serum Laboratories, Melbourne, Australia; protein, 1.4 mg/ml; Der p 1, 66.7 µg/ml, and Der p 2: 75 µg/ml). In patients I to IV RAST inhibition was also performed with 50 µl of a dog dander extract (HAL, The Netherlands; Can f 1, 89 µg/ml; Der p 1 and Der p 2 less than 35 ng/ml) for sera I to IV. Serum from a patient with dog allergy, exhibiting high levels of anti-dog dander IgE but no detectable anti-

TABLE I. Major allergen content of allergen extracts

Allergen extract*	Der p 1 (ng/ml)	Der p 2 (ng/ml)	Der p 1/Der p 2 ratio	Can f 1 (μg/ml)	Can f 1/Der p 1 ratio
A (SPT)	33.4 ± 1.9†	31.3 ± 1.9†	1.07	169.8 ± 12.3†	5,085
B (SPT)	5.1	3.0	1.70	11.1	2,180
C (SPT)	29.6	7.5	3.95	13.3	451
D (SPT)	0.4‡	<0.56‡	>0.16	3.8	9,440
E (SPT)	1.9‡	<1.1‡	>0.76	59.4	31,275
F (RAST)	101.1	72.3	1.40	535.0	5,292
G (RAST)	275.3	334.0	0.82	1,272.6	4,623
M (SPT)	8,751	1,670	5.2	<0.043	<0.00021

*Extracts A to G are dog dander extracts; extract M is a house dust mite extract.

†Mean ± SD for six different batches.

‡Values corrected for concentration (D, 4.5-fold; E, 2.2-fold).

SPT, SPT preparations; RAST, dog dander allergen extracts used in RAST.

HDM IgE in the routine RAST served as a control (patient 0). The serum of this patient was diluted to a concentration eliciting a percentage binding in the concentrated dog dander RAST similar to that of sera from patient I and patients II to IV (labeled serum 0-a and 0-b, respectively).

The sera of patients I and II were tested with a series of diluted HDM extracts bound to solid phase to calculate the concentration of Der p 1 and Der p 2 bound that gave RAST results equal to those obtained with the concentrated dog dander RAST. For this purpose, an HDM (*D. pteronyssinus*)-Sepharose preparation, with known amounts of bound Der p 1 and Der p 2 (6 mg Sepharose/ml; 10.5 μg protein, 500 ng Der p 1, and 560 ng Der p 2 per milligram Sepharose), was diluted in threefold dilution steps with use of glycine Sepharose solution (2 mg/ml).

SPT extracts

Dog dander SPT preparations (A to E) were obtained from five manufacturers (ALK, Copenhagen, Denmark [10,000 BU/ml]; Artu Biologicals, Lelystad, The Netherlands [5 mg/ml]; Bencard, München, Germany [150% CH]; Diephuis, Groningen, The Netherlands [5 mg/ml]; and HAL, Haarlem, The Netherlands [10 mg/ml]). Preparations D and E were also analyzed after concentration (4.5- and 2.2-fold, respectively); by ultrafiltration (Centricon-10; Amicon, Beverly, Mass.).

In addition, a *D. pteronyssinus* SPT preparation (M) was tested in the three major allergen assays as a positive control.

Der p 1 and Can f 1 assays

The Der p 1 and Can f 1 assays used are competitive radioimmunoassays as described previously.⁷ Results are expressed as the mean of two experiments. In brief, 50 μl samples of the SPT preparations were incubated in duplicate for 2 hours with polyclonal rabbit anti-Der p 1 and polyclonal rabbit anti-dog dander antibodies, respectively. Antibodies were bound to solid phase by protein A Sepharose (Pharmacia, Sweden). Affinity-

purified ¹²⁵I-labeled Der p 1 or Can f 1 was added, and the samples were incubated overnight with vertical rotation. After washing, the percentage of radioactivity bound was assessed. Der p 1 concentrations were read from an in-house reference calibrated against the World Health Organization reference (125 pg/U for Der p 1; detection limit, 2.0 ng/ml).^{8,9} Can f 1 concentrations were read from a Food and Drug Administration standard curve (2000 pg/U for Can f 1; detection limit, 3.1 ng/ml).¹⁰

Der p 2 assay

Der p 2 concentrations were determined by using a sandwich-type radioimmunoassay as described before.¹ Results are expressed as the mean of two experiments. In brief, a mix of two human sera with anti-Der p 2 IgE activity was added to 50 μl samples of the various dog SPT preparations in duplicate. Monoclonal anti-Der p 2 antibody coupled to CNBr-activated Sepharose was added, and the samples were incubated overnight with vertical rotation. After washing, ¹²⁵I-labeled sheep anti-human IgE was added, the samples were incubated overnight under vertical rotation, and the percentage of radioactivity bound was assessed. Der p 2 concentrations were read from an in-house reference calibrated against the World Health Organization reference (5 pg/U for Der p 2, detection limit, 2.5 ng/ml).^{8,9}

RESULTS

Clinical data

Of the 1299 patients tested, 328 (25%) had a positive SPT response to dog dander. In 209 (64%) of these subjects, however, no IgE to dog dander was found in the routine dog dander RAST. Of these patients, 158 (76%; 95% confidence interval: 70 to 81) were sensitized to HDM (*D. pteronyssinus*) as indicated by positive results in the HDM RAST. Sensitization to grass pollen was present in 110 cases (53%; 95% confidence interval: 46 to 59)

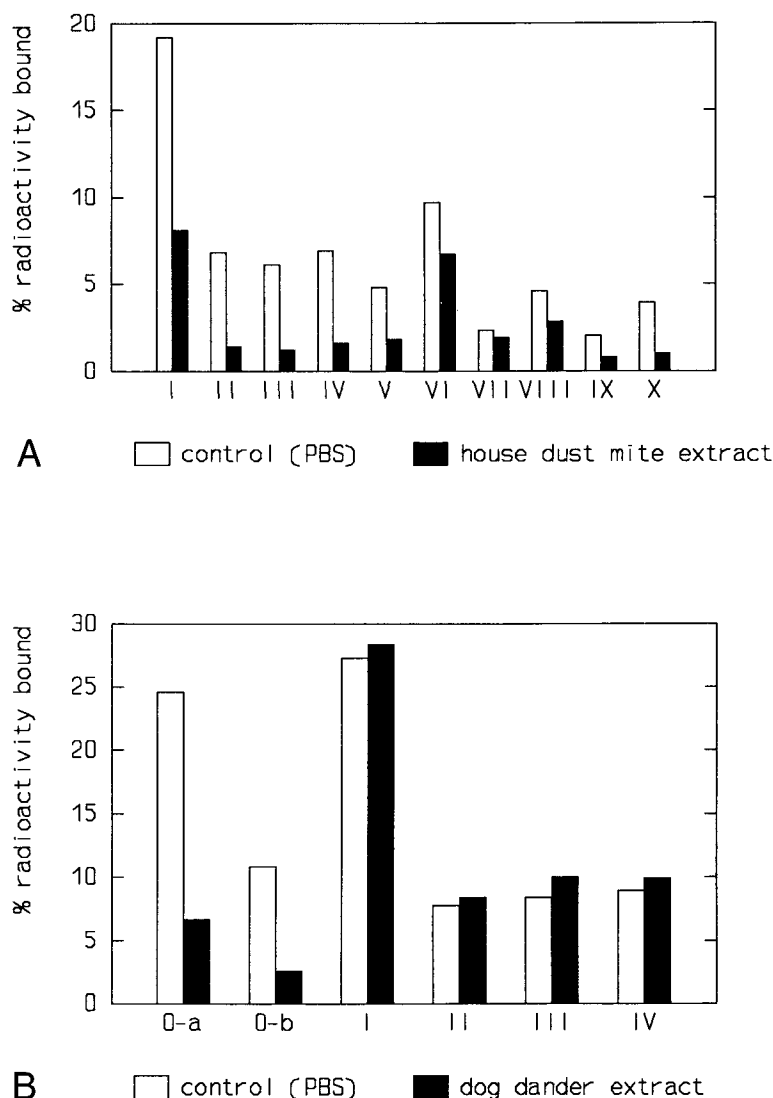


FIG. 1. RAST and RAST inhibition with concentrated dog dander solid phase. **A**, Inhibition of concentrated dog dander RAST with HDM extract. *I* to *X*, Sera of patients *I* to *X* with false-positive dog dander SPT responses. **B**, Inhibition of concentrated dog dander RAST with a dog dander extract. *I* to *IV*, Sera of patients *I* to *IV* with false-positive dog dander SPT responses. *0-a* and *0-b*, Serum of a control patient allergic to dogs, which was diluted to a concentration with binding similar to uninhibited RAST of patient *I* and patients *II* to *IV*, respectively.

and to cat dander in 85 cases (41%; 95% confidence interval: 34 to 48).

With use of a standard questionnaire, animal-related allergic symptoms were documented in 91 (84%) of 108 interviewed patients with allergy to dogs and positive SPT and RAST results with dog dander. In contrast, animal-related symptoms were present in 37 (46%) of 80 interviewed patients who had a potential false-positive response to SPT with dog dander, with no detectable anti-dog dander or anti-cat dander IgE in the routine RAST ($p < 0.0001$, χ^2 test). For comparison, animal-related

symptoms were mentioned by 178 (19%) of the 927 interviewed patients with negative SPT responses to dog or cat dander.

Major allergen content of the allergen extracts

The results of the assays for the HDM major allergens Der p 1, Der p 2, and the dog major allergen Can f 1 are presented in Table I. Contamination with Der p 1 and Der p 2 was demonstrated in six different batches of extract A and in extracts B and C. For extracts D and E, the Der p 1 levels

TABLE II. Results of SPTs with dog dander allergen extracts in four patients sensitized to HDM but not to dog dander

	Mean wheal/flare diameter (mm) of SPTs with extract				
	A	B	C	D	E
Patient 1	4.0/23.5	4.0/20.5	4.5/29.5	2.5/8.0	2.5/2.5
Patient 2	4.25/30.5	3.75/21.5	4.75/28.5	—	2.0/2.0
Patient 3	4.0/23.0	3.75/17.5	3.0/22.5	—	0/0
Patient 4	4.5/24.5	3.75/23.0	3.5/22.0	—	3.0/20.5

Values are mean wheal and flare diameters of duplicate SPTs.

were found to be low and detectable only after 4.5- and 2.2-fold concentrations, respectively (D, 0.4 ng/ml and E, 1.9 ng/ml). Der p 2, however, was not detectable in preparations D and E even after concentration (D, less than 0.56 ng/ml and E, less than 1.1 ng/ml). The calculated ratios of the concentration of Der p 1 to Der p 2 for the different dog dander SPT preparations varied from 1.07 to 3.95.

The concentration of the major dog allergen Can f 1 varied from 3.8 µg/ml for preparation D to 170 µg/ml for preparation A. To allow comparison of the contamination of the dog dander extracts with HDM allergens, the ratio Can f 1/Der p 1 is shown in Table I. This ratio varied from 31,275 for preparation E to 451 for preparation C.

RAST and RAST inhibition

The sera of 10 subjects with potential false-positive SPT responses with dog dander were retested in a RAST with a concentrated dog dander extract (extract F). This extract was identical to the extract used in the SPT and was bound to solid phase at high concentration. With use of this RAST, IgE binding was detected in the serum of all 10 patients tested (median 5.4% radioactivity bound, range 2.0% to 19.2% radioactivity bound). The RAST of these patients was inhibited with use of an HDM extract (inhibition $57.9\% \pm 20.9\%$ [mean $\pm$ SD]; Fig. 1, A). In contrast, no inhibition of the concentrated RAST was found with a dog dander extract in 4 patients tested (patients I through IV; Fig. 1, B). However, RAST inhibition with dog dander extract could clearly be demonstrated in a control patient with high levels of anti-dog dander IgE (sera 0-a and 0-b: $74.4\% \pm 1.6\%$ inhibition, [mean $\pm$ SD]; Fig. 1, B).

The RAST results of patients I and II, with use of the allergosorbent of the concentrated dog dander RAST, were 19.2% and 6.8% of added radioactivity bound, respectively. The concentration of contaminating mite allergens in this RAST

is approximately 0.9 ng Der p 1 and 1.1 ng Der p 2 per test. The concentrations of HDM major allergens bound to the diluted HDM allergosorbent that had equal IgE binding capacity corresponded to 5.8 ng Der p 1 and 6.5 ng Der p 2 per test for patient I and 1.4 ng Der p 1 and 1.5 ng Der p 2 per test for patient II.

Der p 1 threshold in SPT

The median threshold concentration of an HDM extract causing an SPT response with wheal diameter of 3 mm was found to be 5.8 ng Der p 1 per ml (range 3.5 to 20.8 ng/ml) in nine patients highly sensitized to *D. pteronyssinus*.

Dog dander SPTs

Dog dander SPT preparations A, B, and C resulted in clearly positive SPT results in all four patients highly sensitized to HDM but not to dog dander (Table II). SPT extract D was tested in only one patient and did elicit a marginally positive SPT response (mean wheal diameter, 2.5 mm, mean flare diameter, 8.0 mm). There was a statistically significant rank correlation between skin reactivity of the dog dander SPT preparations and the contamination with HDM allergens, expressed as Der p 1 concentration (pooled Kendall- τ test; wheal: $z = 2.77$, $p = 0.006$; flare: $z = 3.38$, $p = 0.0007$). In contrast, no significant rank correlation of skin reactivity with the concentration of Can f 1 was found for the dog dander SPT preparations A to E in the four patients tested (pooled Kendall- τ test; wheal: $z = 0.31$, $p = 0.76$; flare: $z = 0.92$, $p = 0.36$).

DISCUSSION

In this study we demonstrated the contamination of five commercially available dog dander SPT extracts with the HDM (*D. pteronyssinus*) major allergens Der p 1 and Der p 2. The concentrations of Der p 1, Der p 2, and the major dog allergen Can f 1 varied for the extracts tested. In addition,

the ratio of Der p 1 to Can f 1, indicating the relative contamination by HDM major allergens, varied as well.

Minute concentrations of allergen will result in positive skin test responses in subjects highly sensitized to the relevant allergen.¹¹ The Der p 1 concentrations found in the dog dander SPT preparations can exceed the threshold values for positive HDM SPT response that we found in patients with HDM RAST scores of 4+ or 5+. Hence, these extracts elicited false-positive dog dander SPT responses in four house dust mite-sensitized patients having no IgE antibodies to dog dander. In these dog dander SPTs, a significant rank correlation was found between skin reactivity and the Der p 1, but not the Can f 1, concentration of the dog dander extracts tested.

Contamination of an allergen extract used in the RAST will result in detection of IgE only when a sufficient amount of the contaminant is bound to solid phase.¹ This situation is not likely to occur in a standard RAST. For the routine dog dander RAST described in this study, for example, the contamination with HDM allergens resulted in approximately 6.5 pg Der p 1 and 4.6 pg Der p 2 being bound per test. The SPT is, therefore, more likely to show false-positive results because of contaminants than is the RAST.

In the concentrated dog dander RAST, used in this study, the concentration of relevant allergens and irrelevant contaminating allergens per test was increased by approximately 200-fold as compared with the routine dog dander RAST. In the panel of 10 patients with positive SPT responses and negative routine RAST results with dog dander, IgE to the dog dander SPT extract was detected with this concentrated dog dander RAST. These false-positive dog dander RAST results were inhibited by addition of HDM extract but not by addition of dog dander extract. Therefore, the IgE activity of these patients to the dog dander SPT preparation is, at least partially, explained by anti-HDM IgE. In agreement with this explanation is the calculated amount of contaminating Der p 1 and Der p 2 in the concentrated dog dander RAST that resulted in IgE binding equal to a diluted solid phase HDM preparation with similar amounts of Der p 1 and Der p 2 per test.

Skin test preparations of domestic animal dander are extracts of fur from animals held by animal breeders and veterinarians. The housing condition of these animals and the methods of shaving that are used are probably not sufficiently standardized. The allergen extracts can, therefore, contain a mix

of various allergens and other proteins. The ratios of Der p 1 to Der p 2 that we found in the dog hair and dander extracts A, B, and C were comparable with reported values for this ratio found in house dust.¹²⁻¹⁴ This finding suggests contamination of the dog SPT preparations with house dust, which is likely to occur during the housing and handling of the animals or at the time of substrate collection.

The concentration of the dog dander major allergen Can f 1 varied for the five SPT preparations tested. This significant variation in major allergen content of standardized skin test preparations from different manufacturers was reported previously for HDM (*D. pteronyssinus*) extracts.¹⁵

In conclusion, our finding of positive SPT responses with dog dander in patients with no detectable IgE to dog dander can be explained by contamination of a dog dander SPT extract with HDM allergens. False-positive SPT results can have consequences with respect to avoidance measures. Our findings emphasize the need for the accurate analysis of allergen extracts used for skin tests with respect to contamination by other common aeroallergens. The sensitive assays for detection of major allergens described in this study are helpful tools in this respect.

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