

Antigenic/Allergenic Composition of Poodle/Alsatian Dandruff Extract

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Poodle and Alsatian dog dandruff extracts were characterized by crossed immunoelectrophoresis (CIE) and crossed radioimmunoelectrophoresis (CRIE) using sera from 24 individuals clinically sensitive to dogs. By using a system with intermediate gel in immunoelectrophoresis, the content of dander-specific and serum-specific allergens was established. 29 antigens (18 dander-specific and 11 serum-specific) were identified in the mixed breed Poodle/Alsatian dandruff extract. Of these, 24 antigens were radiostained in CRIE. 16 allergens were dander specific and the remaining eight were serum specific. Positive dog dander RAST (e5 and Poodle/Alsatian dandruff extract) results were observed in the tested dog hypersensitive subjects. Our results suggest that the mixture of Poodle/Alsatian dandruff extract may be a suitable preparation for the diagnosis and treatment of dog allergy.

Key words: allergens; CIE/CRIE; Poodle/Alsatian dandruff; RAST.

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Materials of animal origin have been recognized for many years as being responsible for allergic disease in man. Dog allergen extracts prepared for diagnosis of dog allergy are produced from different source materials: whole pelt, skin scrapings, serum, saliva and urine (4, 5, 10, 13, 16, 21, 25). Of these source materials dog hair and dandruff are believed to give the best extract with respect to allergenic composition (4, 9). Studies on the antigenic and allergenic composition of dog hair and dandruff (4, 5, 11, 13), dog saliva (16, 21) and dog urine (8, 10, 16) have been reported.

We have previously shown, by using crossed immunoelectrophoresis (CIE)/crossed radioimmunoelectrophoresis (CRIE) and RAST that dog dandruff might be the best source material for preparation of dog allergenic extracts for diagnosis and treatment of dog allergy (17, 18). In this study, we report a detailed CIE/CRIE analysis of the antigenic and allergenic composition of a Poodle/Alsatian dandruff extract mixture. This two-breed extract was further used to study the IgE responses of a clinically

sensitive patient panel by CRIE and RAST analysis.

MATERIAL AND METHODS

Dog dandruff extracts

Extracts were prepared from Poodle dandruff (lot No. 2180005), Alsatian dandruff (lot No. 2281001) and a mixture of Poodle and Alsatian dandruff (50/50 (w/w) extract). The source material was obtained from Allergon AB, Sweden. Dandruff were obtained from collected poodle hair and alsatian hair, respectively. The hair was processed in acetone. After separation of the hair from the acetone solution, dog dandruff was collected on filter paper and let stand for air-drying. The individual breeds and the two-breed mixture (50/50 extract) were each extracted at a weight volume ratio of 1:100 in 0.1 M sodium hydrogen carbonate buffer, pH 7.8. The extraction was performed whilst stirring in a cold-room (4-8°C) for 18 h. The extract was cleared by centrifugation at 14,000 g

for 30 min and filtered. The crude dandruff extracts were lyophilized.

Antisera

Rabbit antisera to dog dander proteins and dog serum proteins were raised in rabbits (New Zealand White) by multiple intracutaneous immunizations with a crude dog dandruff extract (a mixture from several breeds including poodle and alsatian) and dog serum from several breeds, in Freund's complete adjuvant, essentially as described by Harboe et al. (7). The antisera used were a pool of several bleedings of 3–6 rabbits.

Patient sera

Patient sera were obtained from 24 individuals with clinical history of dog sensitivity. Skin prick tests were also performed with dog dander. The individuals had not been subjected to immunotherapy.

RAST

RAST analyses were performed on the individual patient serum samples with commercially available Phadebas RAST® kits (e1, a cat epithelium disc; e2, a dog epithelium disc; e5, a dog dander disc), Pharmacia Diagnostics AB, Uppsala. RAST analyses were also carried out with an experimental Poodle/Alsation dander disc (50/50 extract) and a dog serum disc. The Poodle/Alsation dander disc and the dog serum disc were coupled analogously to the commercial discs to obtain maximal sensitization.

Protein determination

The total protein content in the dandruff extracts was determined by amino acid analysis (20).

Crossed immunoelectrophoresis (CIE)

CIE was performed on 5 × 7 cm Gel Bond Film (Pharmacia Fine Chemicals, Uppsala) with intermediate gel (2, 3) using 0.025 M TRIS/

Tricine buffer (Bio-Rad), pH 8.6 in 1% agarose (Agarose A, Pharmacia Fine Chemicals) essentially in accordance with the previously described procedure (3, 18).

Samples of 5 µl crude dandruff extract (20 mg/ml) were run in the first dimension electrophoresis at 10 V/cm for 43 min at 15°C. The second dimension electrophoresis was performed at 2 V/cm perpendicular to the first for 18 h using 11 µl rabbit antiserum against dander proteins per cm² gel (upper gel). In the intermediate gel, 5 µl rabbit antiserum against dog serum proteins per cm² gel was used. Cathodic migration was examined by casting an agarose gel with antibodies on the cathodic side of the first dimension gel slab. Identification of individual components was achieved using tandem CIE in accordance with Axelsen et al. (3).

Crossed radioimmuno-electrophoresis (CRIE)

The washed CIE plates were incubated with 0.4 ml of the patients' sera, diluted to 7 ml with CRIE incubation buffer (0.05 mol/l phosphate, pH 7.4, containing 0.9% sodium chloride, 0.3% Tween 20 and 0.3% Dextran 10) for 18 h at 22°C in plastic boxes. After four washings, the CIE-plates were incubated with ¹²⁵I-anti-IgE solution (Phadebas RAST® Isotope Reagent) in 7 ml of CRIE incubation buffer (72,000 cpm/ml) for 18 h at 22°C. After washing with buffer and distilled water four times, the CIE plates were dried and placed on X-ray photographic film for rapid autoradiographic exposure in cassette (at low temperature, -70°C) equipped with an X-ray intensifying screen (22). As a negative control, a pool of individual patient sera with no specific IgE antibodies when tested with RAST (No. 8369, <0.35 Phadebas RAST® Units per ml) was used.

RESULTS

The antigenic content in Poodle dandruff, Alsation dandruff and the mixed Poodle/Alsation dandruff (50/50 extracts) was examined by CIE analysis. Figs. 1A and 1B demonstrate the CIE pattern of Poodle dandruff and Alsation dan-

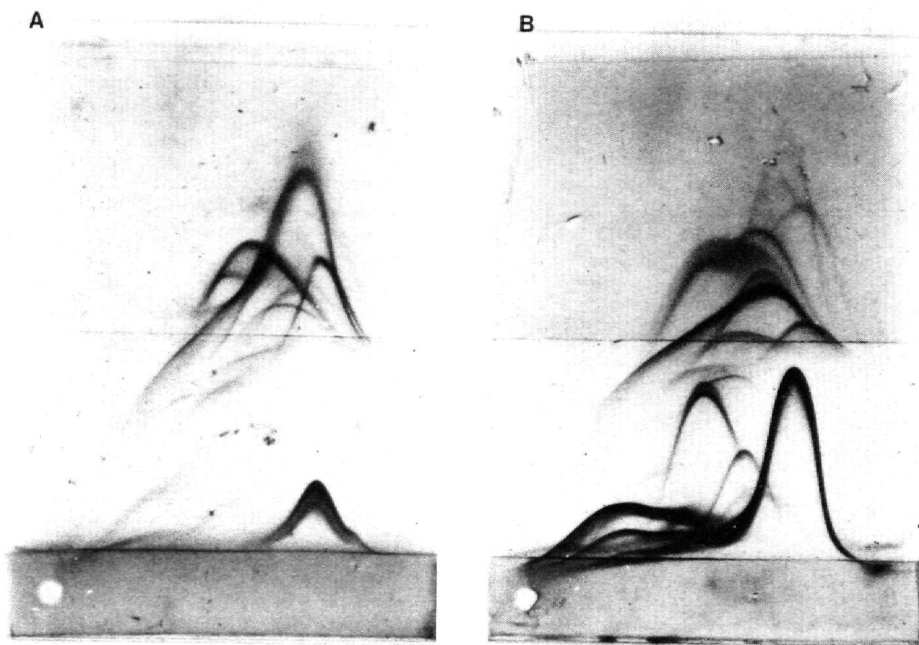


Fig. 1A. Crossed immunoelectrophoresis of Poodle dandruff extract against hyperimmune rabbit antisera, after protein staining with Coomassie Brilliant Blue. 5 μ l of Poodle extract (20 mg/ml) was analysed in agarose electrophoresis (pH 8.6) with 10 V/cm at 15°C for 43 min, followed by electrophoresis perpendicular to the first dimension with 2 V/cm for 18 h in agarose gel (upper gel; rabbit antisera against dog dander proteins, 11 μ l/cm² and intermediate gel; rabbit antisera against serum proteins 3 μ l/cm²). Anode to the right in the first dimension and at top in second dimension electrophoresis.

B. Crossed immunoelectrophoresis of Alsatian dandruff extract against rabbit antisera after protein staining with Coomassie Brilliant Blue. Experimental conditions as described in Fig. 1A. The content of rabbit antisera in the intermediate gel was 5 μ l/cm².

druff extracts against dog dander rabbit antisera after protein staining with Coomassie Brilliant Blue. By using a CIE system with intermediate gel containing rabbit antisera against dog serum components, it was possible to discriminate directly between dog dander specific proteins and dog serum specific proteins in the respective dandruff extracts. 17 antigens (both serum and dander related) were present in the Poodle extract while 24 antigens were demonstrated in the Alsatian dandruff extract. No antigens moved against the cathode under the experimental conditions used indicating that the resolved components were acidic in nature. The CIE pattern of the Poodle dandruff extract

shows a low number of serum-related components compared to the Alsatian dandruff. In contrast, the content of specific dander components appeared to be similar.

Fig. 2A demonstrates the protein-stained CIE pattern with intermediate gel of the Poodle/Alsatian dandruff extract (50/50) after protein staining with Coomassie Brilliant Blue. Fig. 2B is a schematic drawing of the CIE reference pattern of Poodle/Alsatian dandruff extract (50/50). 29 antigens were present in the mixed dog dandruff extract, of which 11 antigens were serum-related and 18 dander-related components. The different antigens were arbitrarily numbered from the anode to the cathode, with

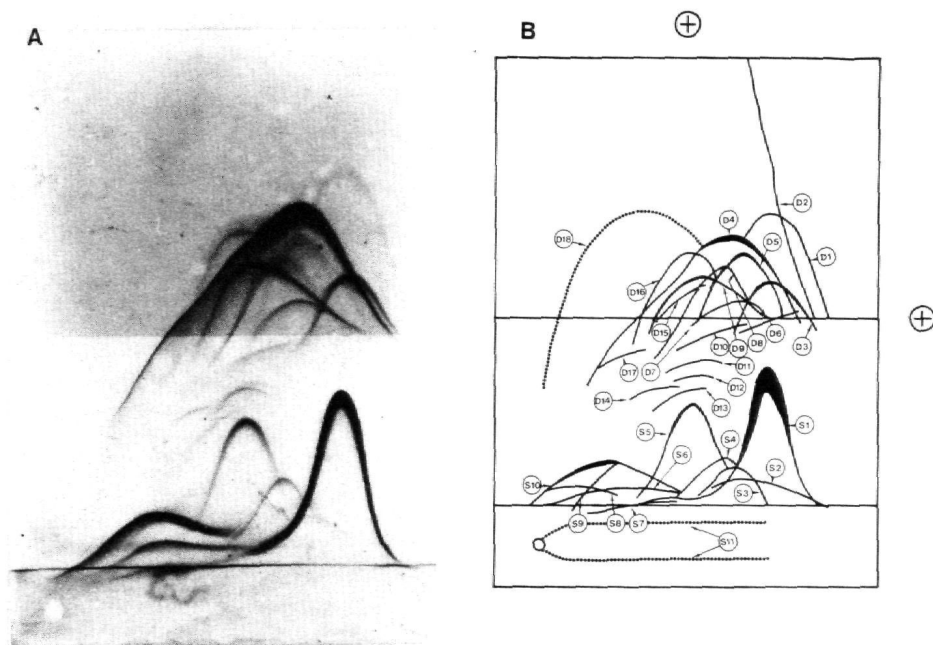


Fig. 2A. Crossed immunoelectrophoresis analysis of Poodle/Alsatian dandruff extract (50/50 w/w) against hyperimmune rabbit antisera after protein staining with Coomassie Brilliant Blue. Experimental conditions as described in Fig. 1B.

B. Schematic representation of the immune precipitates (Fig. 2A), arbitrarily numbered S1–S11 and D1–D18 from the anode (S means serum-related, D means dander-related).

—: sharp visible precipitates; ----: precipitates only visible after radiostaining.

dander-specific antigens numbered D1–D18 and serum-specific components S1–S11. The reference pattern was constructed from several experiments by varying the ratio antigen/rabbit antisera. Immunoprecipitates indicated by dotted lines (Fig. 2B) were only detected after incubation with patient serum and isotope reagent in the CRIE analysis. Two serum-related components were identified by tandem CIE: antigen No. S1 as dog serum albumin and antigen No. S10 as dog serum immunoglobulin G. Similar antigenic patterns were obtained with commercial rabbit antiserum from ALK (Copenhagen).

CRIE analysis of the specific IgE binding in 24 individual sera (with clinical histories of dog allergy) is summarized in Table 1. The results

are expressed in a semiquantitative system (1) based on radiostaining of an X-ray film after exposure for different time periods (1 h to 27 h) corresponding to CRIE class A (dense radiostaining) to CRIE class G (weak radiostaining). Specific IgE binding was demonstrated at varying degrees of radiostaining for 24 different precipitates in the Poodle/Alsatian dandruff extract (50/50). 16 dander-specific allergens and eight serum-specific allergens could be identified. Fig. 3 demonstrates some characteristic radiostained patterns of some patient sera.

By applying a previously described CRIE classification system (18), allergens may be grouped as major, intermediate and minor allergens, depending on their IgE binding ability in CRIE analysis. Antigen No. S1 (albumin)

Table 1

Specific IgE against Poodle/Alsatian dandruff components measured by radiostaining in CRIE analysis. Different amounts of bound specific IgE are shown as hatched squares, symbolising the CRIE classes A–G (Class A, high degree of radiostaining, class G, low degree of radiostaining).

ALLERGOGRAM																															
			S SERUM RELATED ALLERGENS											D DANDER RELATED ALLERGENS																	
PATIENT		ANTIGEN	1	2	3	4	5	6	7	8	9	10	11	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
C R I E C L A S S E S		7307																													
		7308																													
		7309																													
		7310																													
		7311																													
		7312																													
		7315																													
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	A	7321																													
	B	7322																													
	C	7323																													
C	7324																														
D	7328																														
D	7334																														
E	7338																														
E	7341																														
F	7342																														
F	7342																														
G	7630																														
G	7633																														
G	7648																														
G	7656																														
G	8369 (neg. contr.)																														

and No. S10 (gammaglobulin are major allergens present in dog serum. Antigen Nos. D3, D4, D8 and D9 constitute dander-related major allergens not present in the serum. Antigen Nos. S2, D1, D14, D15 and D17 constitute minor allergens. The other allergens identified in Table 1, not belonging to the two extremes, comprise the intermediate allergens.

RAST characteristics of the 24 subjects examined are summarized in Table 2. All sera were analysed with a cat epithelium disc (e1), a dog epithelium disc (e2), a dog dander disc (e5), an experimental Poodle/Alsatian dander disc (50/50) and a dog serum disc (d.s.). 96% of the subjects showed RAST activity class 2–4 for 50/50 discs and 92% showed RAST result class 2–4 for e5 discs.

76% of the subjects gave a RAST result class 2–4 for e1 discs, demonstrating that dog sensi-

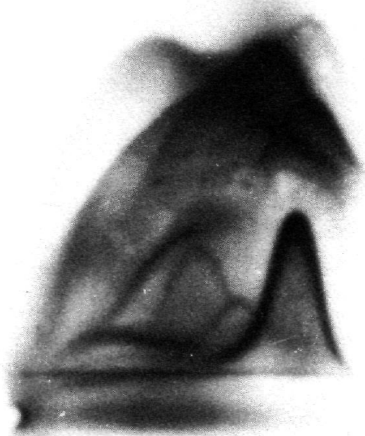
tivity is often accompanied by sensitivity to cats. 88% of the patients showed e2 RAST class 2–4 for e2 discs and more than half of the subjects (60%) gave RAST class 2–4 for (d.s.) discs.

As shown in Table 2, dog dander discs (e5 and 50/50) identified more positive individuals than the dog epithelium (e2) discs. Furthermore, e5 RAST and 50/50 RAST showed very high sensitivity as well as specific RAST activity, which correlated well with clinical history. In addition, e2 RAST also gave two false negative results for patients No. 7338 and No. 7656.

DISCUSSION

The purpose of the present study was to characterize a Poodle and an Alsatian dandruff extract with respect to breed-specificity and further to analyze a Poodle/Alsatian dandruff mixture to

A



B



C



D



Table 2

Allergological characteristics of dog-sensitive patients. Specific IgE antibodies were measured by Phadebas RAST® using cat epithelium (e1) discs, dog epithelium (e2) discs, dog dander (e5) discs, Poodle/Alsatian dander (50/50) discs and dog serum (d.s.) discs.

DISCS	CAT EPITHELIUM	DOG EPITHELIUM	DOG DANDER	POODLE/ALSATIAN DANDER	DOG SERUM
Pat	e1	e2	e5	(50/50)	d.s.
	CLASS	CLASS	CLASS	CLASS	CLASS
7307	4	3	3	3	2
7308	4	4	4	4	0
7309	3	4	4	4	3
7310	3	3	4	4	3
7311	4	4	4	4	3
7312	0	4	4	4	2
7315	4	4	4	4	0
7316	3	3	3	3	1
7318	4	3	4	3	2
7319	4	3	4	3	0
7320	0	4	4	4	0
7321	2	3	3	3	2
7322	2	3	3	4	0
7323	4	4	4	4	0
7324	4	4	3	3	3
7328	4	3	4	3	0
7334	1	0	1	2	2
7338	3	0	3	2	3
7341	3	2	4	4	4
7342	3	3	3	3	3
7630	0	2	2	2	2
7633	3	3	3	3	4
7648	2	4	4	4	4
7656	0	0	3	2	0
8369	0	0	0	0	0

atopic patients using CIE/CRIE and RAST. Fagerberg & Wide (6) proposed the existence of breed-specific components in dandruff from different breeds of dogs using RAST. Blands et al. (4) investigated dandruff extract from six different breeds by CRIE analysis and found breed variations, which they explained to be due to the dander content, i.e. more dander in relation to hair in the short-haired breeds. It has been suspected that adverse reactions to dog dandruff extract have also been breed-specific (14, 15). However, it has been very difficult to verify differences between individual breeds as atopic subjects often develop allergies to animal danders as a group (15, 19) and besides, the extracts used before varied widely in composition (17).

Antigens derived from dog dandruff are acidic in nature as they migrate towards the anode on CIE analysis (Figs. 1 and 2). Our CIE data on Poodle and Alsatian dandruff extracts show that these two breeds contain different

numbers of detectable antigens (17 and 24 respectively) and further that the content of serum-specific proteins is higher in the Alsatian than in the Poodle extract. On the other hand, the content of dander-specific antigens in Poodle extract appears to be higher than in Alsatian extract. CRIE data on Poodle and Alsatian dandruff (unpublished data) are in good agreement with the CIE data presented here, which further supports the existence of breed-specific dog allergens.

In our CIE analysis of the mixture Poodle/Alsatian dandruff extract (50/50) we were able to identify 29 antigens (18 dander-related and 11 serum-related components) by using the intermediate gel technique. Of these, 24 antigens were radiostained in CRIE analysis of a selected patient panel, showing their function as allergens (16 dander-specific and 8 serum-specific). Blands et al. (4) investigated an extract of mixed hair and dandruff from six different breeds by CRIE and they found 25

Fig. 3. X-ray films from CRIE experiments, illustrating the radiostaining pattern of different patient sera (Fig. 3A-C). Exposure corresponding to CRIE class G. Negative control Fig. 3D.

antigens of which some were partially identical. Of these, five allergens were dander-related and the remaining 20 were present in both dander and serum. The difference in the content of identified antigens might be due to the resolution in the CIE/CRIE system used, or possibly to the quality of rabbit antisera. In another study of dog hair and dandruff (12) 20 different antigens were identified, of which 11 were allergens. We identified 24 allergens in our Poodle/Alsatian dandruff extract (50/50), of which at least six allergens fulfilled the criteria for major allergens. Our data are in agreement with the findings of Blands et al. (4), but conflict with the findings of Löwenstein (11, 12), as none of the allergens identified in the latter study were qualified as major allergens.

Some controversial data have been reported in the literature regarding the importance of dog serum albumin as an allergen. Investigations by Yman et al. (25) on dog epithelia extracts suggested that dog serum albumin was an important allergen. This suggestion was confirmed by Blands et al. (4) using CRIE analysis of dog dandruff extracts, and by McLean et al. (13) studying a mixed dog dander extract by skin testing and RAST.

On the other hand, Vanto et al. (23), using isolated dog serum albumin in RAST inhibition studies and skin tests on dog-sensitive patients, found that dog serum albumin reacted as a minor allergen. However, this observation might have been due to a limitation in patient selection, as in our study, using CIE/CRIE analysis with the intermediate gel technique on dog-sensitive individuals, most of the patients reacted with strong radiostaining to dog serum albumin, indicating that dog serum albumin is a potent allergen (Table 1).

RAST data on these dog-sensitive individuals (Table 2) demonstrate that the dog dander RAST discs used (e5 and 50/50) correlated well with each other and were better than dog epithelium for the diagnosis of dog allergy. Previously reported RAST inhibition studies have shown that this Poodle/Alsatian dandruff extract had the same or better specificity and allergenic activity than that of dog saliva (24).

Another interesting observation is that more than 75% of dog-sensitive subjects tested also reacted to cat epithelium (e1 disc). This is in agreement with clinical experience of these individuals with manifested dog allergy (15, 19, 24). Cross-reacting allergens in dog and cat extracts are common and most likely they are both dander-related and serum-related (4, 24).

Our study points to the fact that a mixture of Poodle/Alsatian dandruff extract would give an extract with a balanced content of dander-specific and serum-specific proteins. Such a standardized extract might be a superior preparation for specific diagnosis of dog allergy and for the potential treatment, as patients with dog allergy obviously react to dander-specific allergens as well as serum-specific allergens.

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