

Breed-specific dog-dandruff allergens

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Fifty-one patients with clinical history of dog allergy were skin prick tested with eight individual standardized dog breed-allergen preparations, one mixed breed-allergen preparation (Poodle/Alsatian), dog-serum albumin, and histamine hydrochloride, 1 mg/ml. All extracts were characterized by crossed immunoelectrophoresis and crossed radioimmuno-electrophoresis with a pool of sera from patients clinically sensitive to dog. The dog-breed extracts contained common antigens/allergens, as well as components represented only in one or two dog-breed extracts. The concentration corresponding 1000 BU/ml varied from 16 to 100 µg of protein per milliliter. The sensitivity of skin prick test was 67% to 88% for the various dog breed-allergen preparations, but only 18% for dog-serum albumin. Significant difference between the skin test response to different dog breed-allergen preparations indicating dog breed-specific allergens was obtained in 15% of the patients. There was no significant correlation between skin prick test results and symptoms related to a specific dog breed. (J ALLERGY CLIN IMMUNOL 1988;82:196-204.)

Clinical sensitivity to animal allergens is a frequent and important problem in Western countries among people keeping domestic animals in their homes. Diagnosis and treatment of animal allergy and, especially, dog allergy, has been a major problem, mainly caused by the low and variable potency of the allergen extracts used. Different batches of allergen extracts produced by the same manufacturer, as well as extracts of the same species produced by different producers, have been unsatisfactory, caused by variable composition and unknown allergenic potency.^{1, 2}

The selection of allergen source materials, the pre-treatment of source material, and the extraction conditions used are of fundamental importance for the quality of allergenic preparations (allergenic composition). The variability of dog-allergen extracts manufactured for diagnosis and treatment can be explained by the great number of source materials used: whole pelt, epithelium, hair, dander, serum, and saliva.

Diagnostic studies have suggested breed-specific allergenic structures, based on skin and RAST testing

Abbreviations used

CIE:	Crossed immunoelectrophoresis
CRIE:	Crossed radioimmuno-electrophoresis
SPT:	Skin prick test
PRU:	Phadebas RAST unit
BU:	Biologic unit
C _{hi} :	Allergen concentration inducing a wheal same size as that induced by histamine dihydrochloride (1 mg/ml)
DSA:	Dog-serum albumin

of dog-sensitive patients with different dog-dander extracts.³⁻⁶ However, no breed-specific allergens have so far been demonstrated, most likely because skin scrapings or whole pelt, both dominated by dog-serum proteins,⁷ have been used as source material.

This study presents detailed CIE/CRIE analyses of the antigenic/allergenic composition of eight different dog breed-dandruff extracts with the intermediate gel technique and the diagnostic value of these extracts with SPT and RAST.

MATERIAL AND METHODS

Dog-dandruff extracts

Allergen extracts were prepared from dandruff of eight different dog breeds. The dog-dandruff source materials were obtained from overtly healthy dogs from Allergon AB, Ängelholm, Sweden. The studied dog breeds, the number

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TABLE I. Dog breeds, the number of individual dogs of each dog breed in each batch, and the protein content assayed by amino acid analysis in percent of extracted material

Dog breed-allergen extract	No. of individual dogs	Protein content (% of extracted dry material)
Setter	3	31
Alsatian	4	9
Poodle	20	4
Dachshund	40	10
Labrador	3	13
Collie	7	5
Cocker spaniel	4	27
Golden retriever	6	9

of individual dogs included of each dog breed, and the protein content of each batch of the extracted source materials are presented in Table I. The dandruff was obtained from collected hair of each breed. The hair was separated from dander by processing in acetone and collected on filter paper, air-dried, and finally vacuum dried in the final container. Dog-breed dandruff was extracted in sodium phosphate buffer (pH 7.1) containing 0.9% NaCl or 0.125 mol/L of ammonium bicarbonate (pH 7.8) at a constant weight volume ratio (1:100). The extraction was performed at +4° C for 20 hours, and the extract was cleared by centrifugation and filtration. The solution was thereafter ultrafiltrated on Amicon (Amicon Ltd., Gloucestershire, U.K.) hollow fiber cartridge (nominal cutoff, 5000 daltons) and freeze-dried. The lyophilized dog-breed extract was reconstituted in albumin diluent (human albumin, 0.3 mg/ml, in 0.9% saline), and the protein content of each dog breed-dandruff extract was adjusted to 150 µg of protein per milliliter. DSA was prepared in a concentration of 0.24 mg/ml. The same allergenic material was used for SPT, RAST, and CIE/CRIE analyses.

Patients

Fifty-one allergic patients were selected for the study. There were 31 women and 20 men, age range, 17 to 61 years. They all gave a positive history of dog allergy with symptoms of asthma, rhinitis, and skin symptoms, alone or in combinations (17, 47, and 28, respectively). Forty-seven (92%) of the patients were also sensitive to other allergens.

Two of the patients had been subjected to immunotherapy more than 5 years previously. All patients completed a questionnaire asking about the severity of allergy symptoms and asking if they experienced more or less symptoms after contact with a certain dog breed. Seven pollen-allergic patients with no history of dog allergy served as negative control subjects.

Antisera

Rabbit antisera to dog-dander proteins and dog-serum proteins were raised separately in rabbits, as previously described,⁸ with a mixture of dandruff and serum from all dog breeds.

RAST

Experimental RAST disks were prepared of each individual dog breed, a mixture of Alsatian/Poodle (50/50), and DSA. The coupling conditions were similar to those used for the commercial RAST disks to obtain maximal sensitization. RAST analyses were performed on the individual patient serum with each type of disk with serum undiluted or diluted five or 25 times. Results were expressed in Phadebas RAST units per milliliter.

SPT

SPTs were performed during the pollen-free season according to the method of Pepys.⁹ Each dog-breed extract (150 µg of protein per milliliter) was tested in duplicate on the back of the patient. Histamine dihydrochloride (1 mg/ml) and albumin diluent were used as positive and negative controls, respectively. The wheals were encircled after 15 minutes, and the contours were transferred by means of translucent tape to a record sheet. The mean of the longest and the midpoint orthogonal diameters was used in the calculations. The biologic activity of the various dog-breed extracts, the Poodle/Alsatian mixture, and DSA was determined as the allergen concentration inducing a wheal of the same size as that induced by histamine dihydrochloride (1 mg/ml) (C_{50}) in each patient.¹⁰ The diagnostic capacity of the different dog breed extracts in SPT was evaluated by use of 3 mm mean wheal diameter as cutoff limits.

Protein determination

The total protein content in the various dog breed extracts was determined by amino acid analysis.¹¹

CIE and CRIE

CIE was performed with the intermediate gel technique, essentially as previously described.⁸ Samples of 5 µl of the individual dog-breed extract (5 to 10 µg of protein of the dandruff extract) were run in the first dimension electrophoresis at 10 V/cm for 43 minutes at 15° C. The second dimension electrophoresis was performed at 2 V/cm perpendicular to the first for 18 hours with 20 µl of rabbit antiserum against dog-dander proteins per square centimeter of gel (upper gel). In the intermediate gel, 4 µl of rabbit

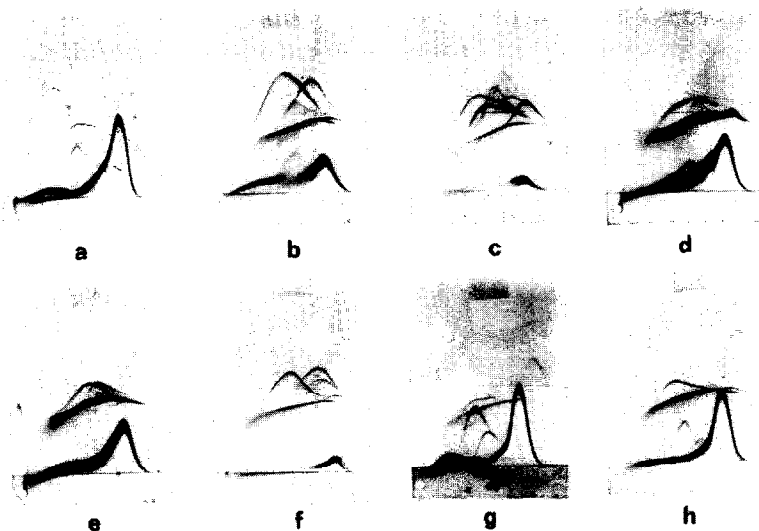


FIG. 1. CIE with intermediate gel of different dog breed-dandruff extracts, 5 μ l: *a*, setter; *b*, Alsatian; *c*, Poodle; *d*, dachshund; *e*, Labrador; *f*, collie; *g*, cocker spaniel; *h*, golden retriever; anode to the *right* in the first dimension and at the *top* in the second dimension. The *intermediate* gel contained rabbit sera against dog-serum proteins, 20 μ l/cm², and the *upper* gel contained rabbit sera against dog-dander proteins, 4 μ l/cm². Protein staining was performed with Coomassie brilliant blue.

antiserum against dog-serum proteins per square centimeter of gel was used.

CRIE analysis was performed as previously described in detail,⁸ with a pool of serum from well-documented dog-allergic patients. For evaluation of the total number of radiostained precipitates of the individual dog breeds, radiostaining corresponding to CRIE class A to G was considered.⁸

Statistics

The statistical significance of the differences between the dog breed-dandruff extracts was tested by the Student's *t* test, regression analysis, and analysis of variance. The individual C_{hi} value was determined as previously described. The median C_{hi} (1000 BU/ml) was determined for each species. For determination of the sensitivity of SPT and RAST, a positive clinical history was considered as true positive disease.

Ethics

This study was approved by the Ethical Committee of the University of Göteborg.

RESULTS

Antigenic/allergenic composition of the individual dog breed-dandruff extracts

The antigenic content of each dog-breed extract was analyzed by CIE with hyperimmune rabbit antisera raised toward a breed mixture of dander and serum components. The protein-stained CIE patterns of the individual dog-breed extracts, by use of the inter-

mediate gel technique, are illustrated in Fig. 1, *a* through *h*.

All dog breed-dandruff extracts demonstrated the presence of both dander- and serum-related antigens. However, the number of protein-stained components differed both with respect to the number of dander- and serum-related antigens as well as the relative content of the individual antigens. Alsatian, Poodle, dachshund, Labrador, and collie (Fig. 1, *b* through *f*) demonstrated several distinctly stained dander antigens, whereas setter (Fig. 1, *a*), cocker spaniel (Fig. 1, *g*), and golden retriever (Fig. 1, *h*) exhibited relatively few protein-stained dander-related antigens. The protein-stained CIE patterns of the serum components were relatively similar for all dog-breed extracts with DSA as the dominating protein. However, the number of serum-related components detected in the CIE patterns of Poodle (Fig. 1, *c*) and collie (Fig. 1, *f*) were definitely lower compared to the other dog-breed extracts.

The allergenic composition of the same dog breed-dandruff extracts assayed by CRIE are illustrated in Fig. 2, *a* through *h*. All dandruff extracts, independent of dog breed, exhibited visible radiostaining after autoradiography for both dander- and serum-related components. Some dandruff extracts (Alsatian, Poodle, dachshund, collie, cocker spaniel, and golden retriever) demonstrated more pronounced radiostaining of both dander and serum components with equal

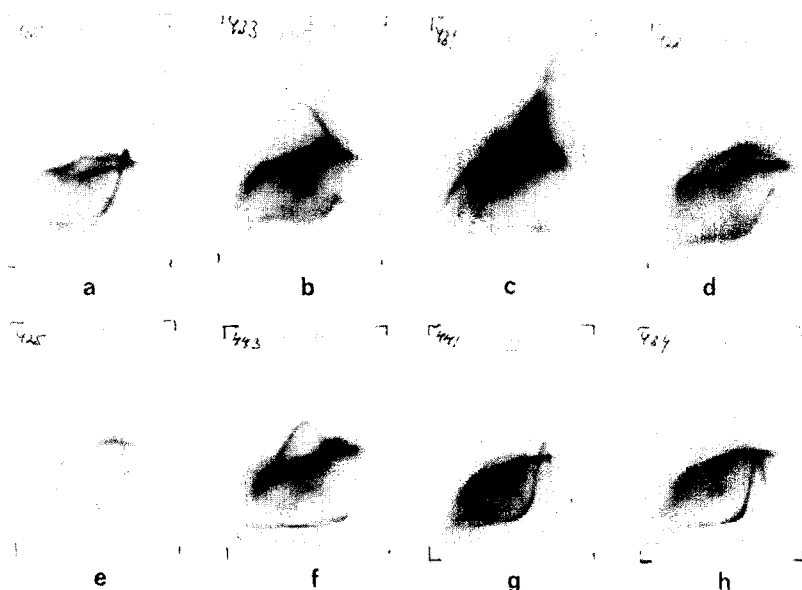


FIG. 2. Photographs of x-ray films from CRIE experiments with a patient serum pool illustrating the radiostaining pattern of different dog breed-dandruff extracts. Exposure time, 27 hours, corresponding to CRIE class G. Experimental notations of the dog-breed extracts same as in Fig. 1.

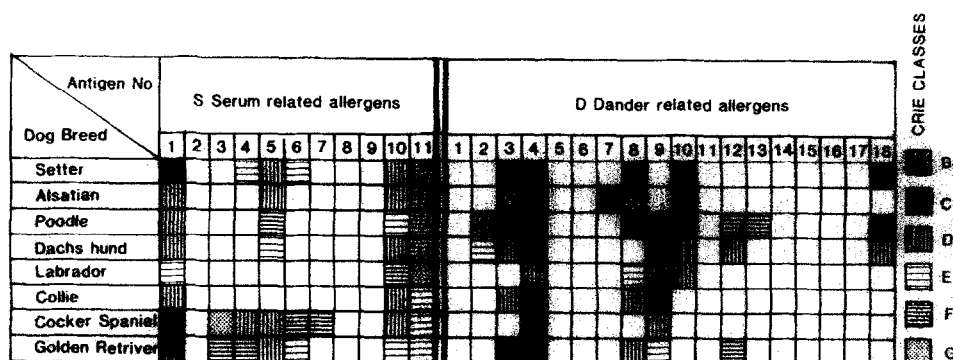


FIG. 3. CRIE radiostaining of different immunoprecipitates in the CIE pattern of individual dog breed-dandruff extracts. The antigens are arbitrarily numbered as in the allergogram presented in reference 8.

amount of protein antigens applied for CIE analysis. Interestingly, Poodle- (Fig. 2, c) and collie- (Fig. 2, f) dandruff extracts demonstrated very strong radiostaining of a great number of dander components and weak radiostaining of the serum components (relatively few identified antigens).

The number of individual allergens in the individual dog-breed extracts was analyzed by CRIE with a representative patient serum pool (Fig. 3). The total number of allergens varied between seven and twelve for the studied dog breeds. Some dog breeds (Labrador and collie) contained only three serum-related allergens in contrast to golden retriever and cocker spaniel extracts, which contained eight and seven, respec-

tively. Cocker spaniel dander contained only two dander-related allergens, whereas Poodle and dachshund contained at least seven identifiable dander allergens.

Specific IgE assayed by RAST

The individual patient serum was examined by experimental RAST disks. Thirty-five patient sera (74%) were RAST positive for at least one dog breed-dandruff allergen of the 48 patient sera analyzed by RAST (Fig. 4).

The uptake on RAST disks was significantly higher for the DSA RAST-positive sera. The same pattern was found for all species and was most pronounced

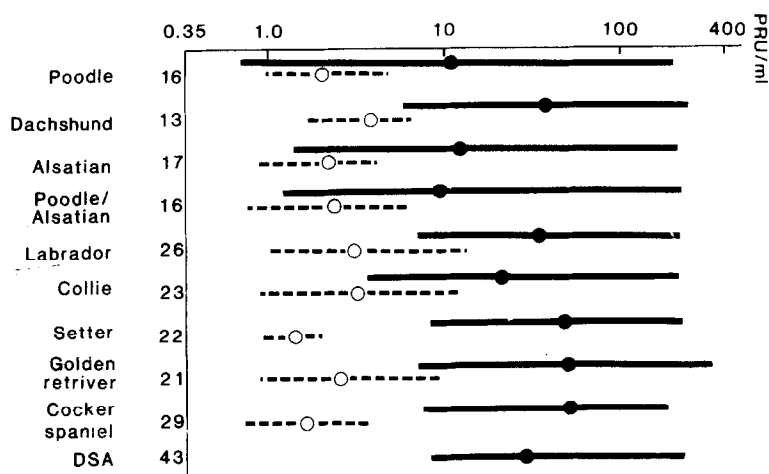


FIG. 4. Specific IgE measured with RAST and expressed as Phadebas RAST units for patients with dog allergy (N = 48) and control subjects (N = 7). Different dog breed-dandruff extracts were coupled to solid phase; median and 95% confidence limits for DSA RAST-positive patients (—●—, N = 7); DSA RAST-negative patients (---○---, N = 43). Patients with negative RAST (<0.35 PRU/ml) are indicated in the figure for each dog breed.

TABLE II. RAST sensitivity in 48 patients with different dog breed-dandruff extracts and DSA

Dog-allergen extract	% RAST positive
Golden retriever	58
Dachshund	74
Poodle	68
Poodle/Alsatian	72
Setter	52
Alsatian	66
Collie	54
Labrador	52
Cocker spaniel	42
DSA	14

Percentage of individuals with positive RAST to >0.35 PRU/ml.
All control patients (N = 7) were negative in RAST.

for those that demonstrated a high ratio of serum to dander components. The control patients were all RAST negative, i.e., demonstrated no circulating-specific IgE.

Among the DSA RAST-negative patients (Fig. 4), the lowest uptake was obtained for setter and cocker spaniel, and the highest for dachshund, collie, and Labrador. In DSA RAST-positive patients, the lowest uptake was obtained for Poodle, Alsatian, and the Poodle/Alsatian mixed disks, whereas golden retriever, setter, and cocker spaniel demonstrated the highest uptake.

The percentage of the individuals with posi-

tive RAST (RAST class 1 or higher, i.e., ≥ 0.35 PRU/ml) for the individual dog breed extract, the Poodle/Alsatian two-breed mixture, and DSA is presented in Table II. The percentage of patients sensitive to the individual dog-breed extract varied between 42% to 74%; cocker spaniel demonstrated the lowest uptake and dachshund, the highest. The two-breed mixture (Poodle/Alsatian) elicited a positive RAST in 72% of the analyzed patients. Only 14% of the analyzed individuals were positive to DSA.

SPT

Results of skin prick testing with the individual dog breed-allergen preparations at a protein concentration of 150 μ g/ml are illustrated in Fig. 5; 50/51 patients (98%) were positive (>3 mm wheal diameter) in SPT to at least one of the eight different dog-allergen preparations. Thirty-four patients (67%) were SPT positive to all eight dog breed-allergen preparations, and 43/51 patients (84%) were SPT positive to the Alsatian/Poodle-dander mixture, whereas only nine patients (18%) were SPT positive to DSA.

No patient was SPT positive to DSA alone. One patient was negative in SPT as well as RAST for all tested allergen preparations, although she had moderate asthma and rhinitis in contact with dogs. Among the negative control patients, 16 skin tests (23%) elicited a positive SPT reaction (≥ 3 mm mean wheal diameter) to at least one of the dog breed-allergen preparations or DSA.

The sensitivity of the SPT/cutoff wheal diameter, 2 and 3 mm, respectively, with the individual dog

TABLE III. The sensitivity of the SPT with different dog breed-allergen preparations and DSA in 51 dog-allergic patients

Dog-allergen extract	Cutoff wheal diameter	
	3 mm	2 mm
Poodle	84	92
Dachshund	88	92
Alsatian	84	86
Poodle/Alsatian	86	94
Labrador	83	92
Collie	84	92
Setter	86	98
Golden retriever	88	92
Cocker spaniel	82	90
DSA	18	24

Percentage of individuals with positive skin test results are presented in relation to 2 and 3 mm mean wheal diameters (cutoff limits).

breed-allergen preparations, the dander mixture, and DSA is presented in Table III in detail.

The Alsatian/Poodle-dander mixture elicited a positive SPT in 48/51 (94%) (≥ 2 mm mean wheal diameter). Only one dog breed-allergen preparation, setter, elicited positive SPT in more patients (49/51). In contrast, setter elicited also positive SPT in some control patients, demonstrating that this individual dog-breed preparation was not optimal in discriminating between dog-allergic and nondog-allergic individuals.

No difference was found in skin response to the different dog breed-allergen preparations; 67% to 88% of the tested patients were SPT positive to the different preparations. There was no significant correlation between the degree of skin sensitivity and the patient's opinion of severity of symptoms to a certain dog breed. However, the skin reaction was significantly larger in patients with severe dog allergy, compared to those with moderate or mild symptoms. The difference in size between the largest and smallest wheal induced by individual dog-breed extracts was compared for each individual patient (Fig. 6). Eight patients (15%) demonstrated a considerable difference in wheal diameter, up to three to four times, to certain dog breed-allergen preparations ($p < 0.01$). The dog breed-allergen preparations that elicited significantly smaller or larger wheals were different for different patients.

The biologic activity, as expressed by the range of C_{hi} values, the median C_{hi} value (1000 BU/ml) with 95% confidence limits of the individual dog breed-

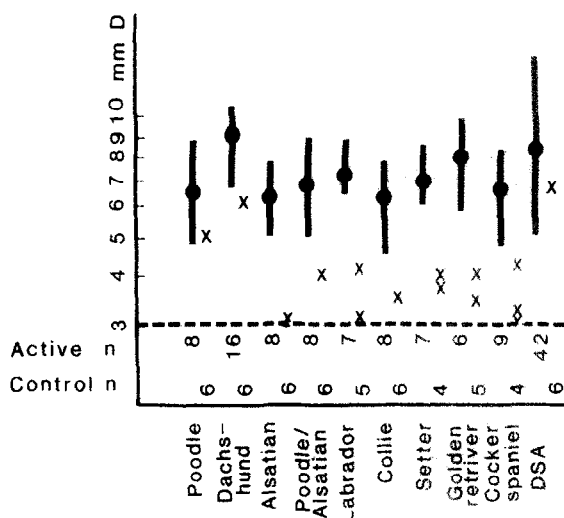


FIG. 5. The median (●) and 95% confidence limits (—) for the wheal diameter with the SPT method with different individual dog breed-dandruff extracts, Poodle/Alsatian and DSA in dog-allergic patients ($N = 51$) and control subjects ($N = 7$). Active and control patients eliciting a wheal diameter < 3 mm are indicated by numbers. Control patients eliciting a wheal diameter > 3 mm are indicated by x.

dandruff extracts, and the two-breed mixture (Poodle/Alsatian), is illustrated in Fig. 7.

The median values are located in the range 16 to 90 μ g of protein. Collie demonstrated the lowest activity, 90 μ g of protein per milliliter, whereas dachshund demonstrated the highest, 16 μ g of protein per milliliter.

DISCUSSION

Previous reported studies of dog sensitivity have mainly focused on the large variation in potency of extracts used and the variations in the relative concentration of antigens and allergens.^{1, 2, 7, 12} The well-known clinical experience that dog-allergic patients tend to react to only some dogs and not to other dogs has not been elucidated or related to the known variations of dog allergens. The purpose of the present study was to characterize individual dog breed-dandruff extracts with defined protein concentration with respect to breed-specific antigens/allergens with CIE/CRIE and to relate the skin reactivity of these dog-allergen extracts to the clinical history in dog-sensitive individuals. In an earlier study, we demonstrated the detailed characterization of Poodle, Alsatian, and the two-breed mixture, Alsatian/Poodle, with immunoelectrophoretic methods.⁸ CIE analysis demonstrated the presence of 29 antigens, and several of the identified antigens/allergens in the two-breed mixture could also be identified in the other six in-

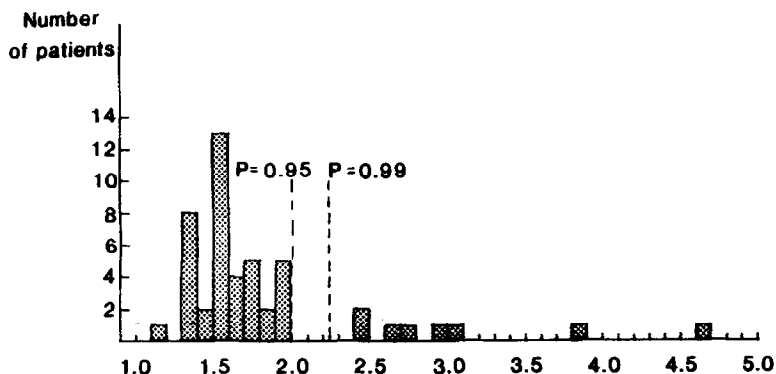


FIG. 6. SPT results with different dog breed-dandruff extracts and DSA. The variation between the size of the largest and smallest wheal is expressed as a ratio:

$$R = \frac{\text{Largest diameter}}{\text{Smallest diameter}}$$

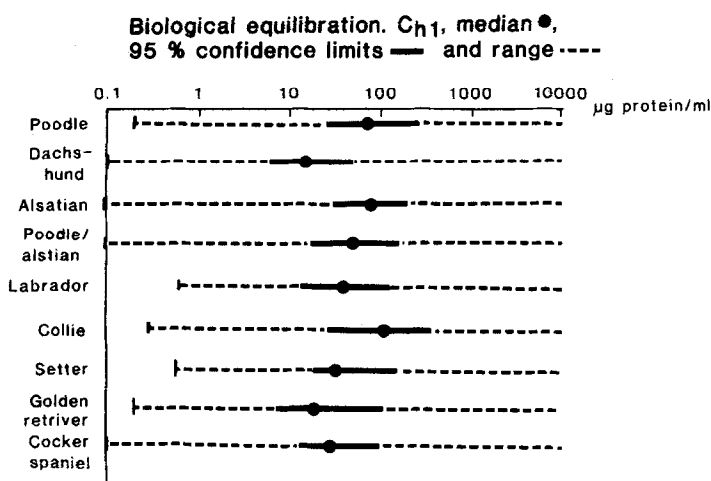


FIG. 7. Biologic activity of the individual dog breed-dandruff extracts and the Poodle/Alsatian mixture, as determined by SPT. The median, C_{H1} (1000 BU/ml) (●), 95% confidence intervals (—), and the range for C_{H1} (---) are presented. The amount of allergenic material in micrograms of protein per milliliter is illustrated on the x axis.

dividual dog-breed extracts included in this investigation. A few allergens could only be identified in one or two dog breeds (Fig. 2), whereas most identified allergens appeared to be common for several of the dog breeds studied.

These findings support the earlier proposals of the existence of common clinically important dog allergens.^{7, 13-16} We have previously identified dog albumin (AgS1) and a dog-dander component (AgD4) as major allergens in Poodle- and Alsatian-dandruff extracts,⁸ and these allergens were also easily identified in the other dog-breed extracts studied (Fig. 2). By use of CRIE analysis, Blands et al.⁷ identified two major allergens (DSA and a dander-related component)

among 21 antigens in a mixed dog hair and dander extract. Most likely, these allergens correspond to our identified AgS1 and AgD4. Furthermore, these authors could not detect any breed-specific allergens in their CRIE system, which is in contrast to our findings with the used intermediate CRIE gel technique. Obviously, the selection of the source material is very important. Serum proteins from skin scrapings dominated the dog-breed extracts used in their experiments and thus might mask the weakly radiostained breed-specific dander components.

All the patients studied except one demonstrated a positive SPT to at least two of the dog breed-dandruff allergen preparations with adjusted protein concentra-

tion. In agreement with previous observations,³⁻⁶ there was an individual variation in skin sensitivity of patients to different dog-breed extracts. However, no correlation was found to the patients' own opinion of allergy caused by a specific dog breed.

The biologic activity, expressed as the median C_{hi} (1000 BU/ml) of the dog-breed extracts, varied from 16 to 90 μ g of protein per milliliter. However, no statistical difference in biologic activity could be detected. The evaluated median C_{hi} (1000 BU/ml) concentration was slightly higher for the two-breed mixture (Poodle/Alsatian) compared to the concentration previously found in the biologic equilibration of the two-breed mixture (9 μ g of protein per milliliter). The reason for the discrepancy might be that the two-breed mixture used in this study was an experimental batch.¹⁰

DSA has been suggested to be an important allergen in patients with dog allergy based on the use of RAST and CRIE.^{7, 9, 11, 16, 17} By contrast, studies based on RAST and SPT have demonstrated that only a minor number of dog-allergic patients gave a positive response to DSA, that is, 10% in RAST and 20% in SPT.¹⁵ The results from the present study are in accordance with these figures, since only 14% of the patients were RAST positive and 18%, SPT positive to DSA. For the DSA positive patients, DSA appeared to be the dominating allergen, since there was a clear correlation between the skin response to DSA and the content of DSA in the dog-allergen preparations.

Positive SPT and clinical history demonstrated good agreement; 67% to 88% of the patients were SPT positive to the dog-breed extracts used. In the RAST analyses, the breed variation was more pronounced; 42% to 74% were RAST positive, supporting a difference in allergenic composition between the breed preparations. Similar differences in RAST results with different dog-allergen preparations have been demonstrated when dog-epithelium and dog-dander allergens are compared.^{13, 15, 18} Some of the negative control patients also gave positive SPT for the tested dog breed-allergen preparations (Fig. 5). This might be due to the atopy among these pollen-allergic patients and a suspected latent dog allergy. All negative control patients also demonstrated negative RAST.

In the present study, we used well-defined dog breed-dandruff allergens and DSA adjusted to contain the same amount of protein independent of dog breed. The results point to the fact that the main source of dog allergens derives from dog dandruff and only a smaller part from dog serum. Published studies have also suggested that dog-allergen extracts dominated by serum components demonstrate less sensitivity¹⁹

than those containing a balanced mixture of dander and serum proteins.

The source of raw material is important because skin scraping will produce many serum components, whereas epithelium components dominate in dandruff preparations. This might explain the bad quality in several commercial dog-allergen extracts. It thus appears reasonable to combine selected dog breeds²⁰ to elicit a well-balanced dog-allergen extract with dander- and serum-related components, as exemplified with the two-breed mixture of Poodle and Alsatian.⁹ The data presented in this study demonstrate that the two-breed mixture had a high diagnostic efficacy in dog-allergic patients compared to the individual dog-breed extracts.

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Alveolar macrophage immunosuppression is maintained in rabbit models of hypersensitivity pneumonitis

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In established experimental models of hypersensitivity pneumonitis and, perhaps, in exposed, asymptomatic humans, continued aerosol exposure to protein antigen results in waning disease and a state of desensitization. The mechanisms causing such unresponsiveness are not well understood, but a possibility is enhanced immunosuppression by alveolar macrophages or other bronchoalveolar cells. Similarly, a loss of immunosuppressive function could result in the appearance of alveolitis. We therefore compared the ability of bronchoalveolar lavage (BAL) cells to augment or suppress antigen-specific lymphocyte blastogenesis in rabbit models of acute and chronic hypersensitivity pneumonitis, desensitized animals, and control animals. We found that BAL cells from all treatment groups suppressed antigen-specific lymphocyte blastogenesis at BAL:lymph node cell ratios of 1:1 to 1:8. BAL cells from some animals were suppressive at high BAL concentrations and, at lower concentrations, augmented the blastogenic response. Additional studies revealed no significant differences in the ability of mitomycin C-treated BAL cells to suppress or augment autologous lymphocyte blastogenesis at any ratio tested. Low-density, macrophage-enriched BAL cells obtained by Percoll fractionation maintained suppressive function. Addition of indomethacin to cultures only partially abrogated BAL-mediated suppression of antigen-specific blastogenesis. We conclude that the development of alveolitis in this model cannot be attributed to loss, nor can desensitization be explained by augmentation, of alveolar macrophage immunosuppressive function. (J ALLERGY CLIN IMMUNOL 1988;82:204-12.)

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The macrophage plays a prominent role in host defense as a component of the nonspecific immune system involved in phagocytic removal of foreign material. In addition, cells of the monocyte/macrophage series are involved in specific immunity through antigen presentation to T-lymphocytes^{1,2} and produce soluble factors, such as interleukin-1 that augment T cell proliferative responses³ and promote B-lymphocyte proliferation and differentiation.⁴ Mac-