

Skin test reactivity to dog-derived antigens

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Summary

Skin test reactivity to canine antigens was studied by testing atopic patients and veterinarians using a commercially prepared mixed-dog epithelial antigen and breed-specific antigens including dander extracts, serum and urine, obtained from thirty-one different pure-bred dogs. Increased skin test reactivity was noted using breed-specific antigens as compared to the mixed-dog commercial screening extract. Variation in skin test responsivity related to specific breed antigens was also noted. The results suggest that skin tests using canine urine and serum antigens, in addition to the conventional dander antigens, may be useful in the diagnosis of hypersensitivity to dogs.

Introduction

Hypersensitivity to companion animals is an important problem in clinical allergy. While sensitivity to dogs is generally attributed to inhalant epithelial proteins, in actuality mammalian allergens can be derived from a wide variety of non-dander animal tissues including saliva, serum, urine, tears, amniotic fluid and semen (Ohman, 1978). Furthermore, recent studies have indicated that there may be some structural differences between dander allergens of different breeds of dogs (Berrens, Henocq & Koers, 1974; Blands, Lowenstein & Weeke, 1977; Fagerberg & Wide, 1970). Successful diagnosis and therapy of hypersensitivity to animals is dependent on the use of allergens which are relevant to the patient's particular clinical sensitivity. Accordingly, we have attempted to establish the relevancy of allergens derived from the dander, urine and serum of various pure-bred canines in the diagnosis of hypersensitivity to dogs, and to study possible differences in skin reactivity based on breed.

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Table 1. Canine antigens used for skin testing

Breed	Dander*	Serum	Urine
Mixed-dog dander (Greer)	+		
Siberian husky	+	+	+
Springer spaniel	+	+	+
Old English sheepdog	+	+	+
Dachshund	+	+	+
Schipperke	+	+	+
Bichon frise	+	+	+
Boston terrier	+	+	+
German shepherd	+		
Labrador retriever	+	+	
Basset hound	+		
Brittany spaniel	+		
Wirehaired terrier	+		
Cocker spaniel	+		
Shih Tzu	+	+	+
Malemute	+	+	+
Great Dane	+	+	
Blue Dane	+	+	
Saint Bernard	+	+	+
Miniature schnauzer	+		
Golden retriever	+		
Airedale	+		
Welsh terrier	+		
German shorthaired pointer	+	+	+
Shetland sheepdog	+	+	+
Chesapeake Bay retriever	+	+	+
English setter	+	+	+
West Highland white terrier	+		
Collie	+		
Irish setter	+		
Keeshond	+		
Weimaraner	+	+	+
Puli	+	+	+

* Used in w/v concentrations ranging from 1:10 to 1:50, depending on amount of raw antigen available.

Materials and methods

Antigens (Table 1)

Epithelial extracts. Hair and dander samples obtained from pelts of thirty-one breeds of dog. Recognized as pure-bred by the American Kennel Club (1976), were defatted in two to three volumes of acetone at room temperature. Following removal from the defatting solution, the epithelial material was air-dried, finely chopped with scissors and weighed. Antigen mixtures were prepared on a weight per volume (w/v) basis (range 1:10 to 1:50, depending on the amount of material available) by extraction with phosphate-buffered saline in capped flasks on a mechanical rotator/shaker in the cold at 4°C for 48 hr. Clarification by preliminary filtration (Whatman Filter Paper No. 2) was followed by sterilization with filtration (Millipore Swinnex Filter 0.45 µm) into

sterile-capped vials for refrigerated storage prior to use. In addition to the pure-bred extracts prepared in our laboratories, a commercial mixed-dog (mongrel) dander extract (Greer Laboratories, Lenoir, NC) was utilized in this study.

Serum. Whole blood was obtained from nineteen different pure-bred dogs aseptically by venipuncture and collected in sterile tubes. After clotting of the blood, serum was separated and transferred by sterile pipette in aliquots to sterile tubes and stored at -70°C . Prior to use, serum was thawed, sterilized by filtration (Millipore Swinnex Filter $0.45\text{ }\mu\text{m}$) and stored in capped sterile vials under refrigeration at 4°C .

Urine. Urine samples from sixteen different pure-bred dogs were collected either aseptically by cysticentesis or by manually expressing the bladder into clean containers. After sterilization (Millipore Swinnex Filter, $0.22\text{ }\mu\text{m}$) urine samples were stored in sterile vials at 4°C .

Prior to use in skin testing, aliquots of urine and serum samples were placed in sterile dropper bottles to which 4% phenol was added as a preservative.

Sterility testing

After filtration, aliquots of dander, urine and serum antigens were removed for sterility testing. Inoculations into thioglycollate and BHI broth media were incubated for 72 hr to determine the possible presence of anaerobic and aerobic organisms. Samples were also inoculated in Sabouraud's agar to determine mycotic growth. No bacteria or moulds could be detected in any sample.

Test population

The fifty-three test subjects were composed of two groups: (1) Twenty-three human atopic patients who had previous demonstrable immediate weal and flare (2+, or weal between 5 and 8 mm or stronger) prick reactions to mixed canine dander allergens who were clinically sensitive to dogs and had no immunotherapy with that antigen, and (2) thirty veterinarians engaged in small animal practice in which they had daily dog contact. These individuals claimed to have clinical allergic respiratory symptoms on exposure to dogs. Six of these veterinarians had received immunotherapy (from 4 weeks to 1 year) to the particular animal dander to which they claimed sensitivity. Results of any previous skin testing were not available in this group.

Test subjects furnished a personal medical history including their allergic illness, symptomatology, family history of atopy, medications and animal exposure.

Prior to skin testing an informed consent was obtained from each subject.

Control subjects

These subjects were four known non-atopics without significant respiratory symptoms and were tested similarly.

No subject had received any anti-allergy preparation for 48 hr prior to the test.

Skin tests

Prick tests were applied to the backs of the subjects using dander, serum and urine antigens and a diluent control. Weal and flare reactions were measured after 20 min, and the results recorded as 0, 1+, 2+, 3+ or 4+ according to the intensity of the reaction. A reading of 2+ (weal 5–8 mm in diameter, erythema greater than 15 mm in diameter) was regarded as the minimum positive test.

The control extract solution consisted of phosphate-buffered saline.

Results

The diagnostic efficacy of commercial screening extract

With the commercially mixed-dog dander antigen as a screening skin test, only three of the thirty (10%) veterinarians demonstrated a positive response, while eight of the twenty-three (35%) clinically atopic patients responded positively to the prick test. Control extract tests were negative. All subjects had histories suggestive of dog sensitivity.

When the same two groups were tested with breed-specific dander, serum and urine antigens, nineteen of the thirty (63%) veterinarians and twenty-two of the twenty-three (96%) atopic patients showed a positive response to at least one of the breed-specific antigens. Every subject who reacted positively to the commercial screening extract had a positive response to at least one dander, one serum and one urine from the breed-specific antigen group. The proportion of positive responses of the total tests for each subject group and each type of antigen is displayed in Table 2. In every instance, the veterinarians and patients who showed a positive response to the commercial screen were more reactive to the breed-specific tests, indicating reactivity to a greater multiplicity of dog breeds than could be evoked with the commercial extract alone.

Among the forty-two subjects who did not react to the mixed-dog commercial screen, ten of the fifteen patients (67%) and nine of the twenty-seven (33%) veterinar-

Table 2. Positive skin test results with dander, serum and urine antigens in fifty-three subjects

Antigen type	No. of antigens	Veterinarians (30)		Atopic patients (23)	
		Screen*-(27) (%)	Screen+(3) (%)	Screen-(15) (%)	Screen+(8) (%)
Dander	33	6.9	34.4**	11.5	35.5**
Serum	19	52.2	58.8	28.6	93.4**
Urine	16	16.7	57.1**	39.8	57.0**

* Screen = tests with commercial mixed-dog dander extract.

** Significant difference between screen (+) and screen (-) ($P < 0.01$).

Number of subjects in parentheses.

Table 3. Positive skin tests to breed-specific antigens in forty-two subjects with negative skin tests to commercial mixed-dog dander antigen

Screen*-negative subjects (n=42)	Positive response with breed-specific antigen		
	Dander (%)	Serum (%)	Urine (%)
Atopic patients (n=15)	67	47	80
Veterinarians (n=27)	33	41	44

* Screen = commercial mixed-dog dander antigen extract.

Number of subjects in parentheses.

ians had a positive response to the purebred dander antigens (Table 3). The commercial dander screen confirmed only eleven of forty-one responders who had been identified by the breed-specific antigens. No subjects were detected by the screen who had not been identified by the breed-specific antigens. Of these forty-one responders, the commercial screen was reactive for three of the nineteen (16%) veterinarians and eight of the twenty-two (36%) atopic patients.

A comparison of the relative diagnostic efficacy between dander, urine and serum

A comparison of skin test responses to the three types of antigenic materials (canine dander, urine and serum) in dog-sensitive individuals is shown in Table 4.

Six of the nineteen veterinarians responded to only one type of antigen: three to serum only, and three to urine only. Among the atopic patients, four out of twenty-two responded to only one type of antigen, one to dander only, one to serum only, and two to urine only. The use of serum only as a test antigen would have missed skin reactivity in four of nineteen veterinarians and six of twenty-two patients, or approximately 25% of the total responder population of forty-one. The utilization of a single type of antigen (dander, urine or serum) only would have therefore failed to detect 15 to 29% of the forty-one positive responders on skin test. However, the use of serum and urine antigens in combination would have identified forty of the forty-one (98%) positive responders. The combined use of dander plus urine antigens or dander plus serum antigens would have had a detection rate of 86% and 87%, respectively.

The distribution of the positive test responses to the three types of antigens are shown in Table 5. A positive (2+ or greater) response to as many as twenty danders was seen in the case of a single patient. With serum antigens, fifteen of the responders had positive responses simultaneously to sixteen or seventeen of the breed-specific serum antigens.

Evidence for allergenicity by breed

Questionnaire responses indicated that a number of individuals believed they were

Table 4. Skin test responses to canine dander, serum and urine antigens in forty-one subjects claiming allergy to dogs

Skin test response	Veterinarians (n = 19)	Atopic patients (n = 22)
Negative to <i>all</i> dander	7	4
Negative to <i>all</i> serum	4	6
Negative to <i>all</i> urine	4	2
Responsive to dander <i>only</i>	0	1
Responsive to serum <i>only</i>	3	1
Responsive to urine <i>only</i>	3	2
Responsive to <i>one or more</i> dander	12	18
Responsive to <i>one or more</i> serum	14	15
Responsive to <i>one or more</i> urine	15	20
Responsive to a dander-serum combination	16	20
Responsive to a dander-urine combination	16	21
Responsive to a serum-urine combination	18	22

Number of subjects in parentheses.

Table 5. Distribution of positive skin test responses to breed-specific canine antigens in forty-one subjects

No. of positive tests* observed	Subjects reacting to breed-specific antigens		
	Dander (<i>n</i> = 13)	Sera (<i>n</i> = 17)	Urine (14) (<i>n</i> = 14)
0	11	12	6
1	4	3	4
2	4	1	2
3	4	0	4
4	3	0	3
5	0	0	2
6	2	0	5
7	0	2	3
8	1	0	1
9	4	2	2
10	1	2	3
11	1	0	0
12	1	0	4
13	1	1	1
14	1	1	0
15	1	1	—
16	1	5	—
17	0	10	—
18	0	—	—
19	0	—	—
20	1	—	—
21-31	0	—	—

* Positive test = 2+ weal and flare or greater.
Number of antigens used in parentheses.

more sensitive to specific breeds of dogs. In the case of sixteen persons who claimed greater sensitivity to a specific breed and were tested with antigens for that breed, nine demonstrated a positive skin response to the dander, serum or urine of that particular breed.

The relative allergenicity of the breed-specific antigen types is shown in Fig. 1. Each curve represents the estimated probability distribution of responses to a particular antigen class. If there were no selective antigenicity, subjects who responded to one antigen would respond to all the antigens in that class. This is the expected response curve in Fig. 1. The observed response curves were compared as to the probability of responding to *X*% or more of the breeds given one response:

$$\text{Probability of responding to } X\% \text{ or more of the breeds} = \frac{\text{no. responding to } X\% \text{ or more}}{\text{no. responding to at least one}}$$

Using the curves to estimate the probability of responding positively to 50% or more of the tests, the probability is approximately 70% for serum, 40% for urine, and 6% for dander.

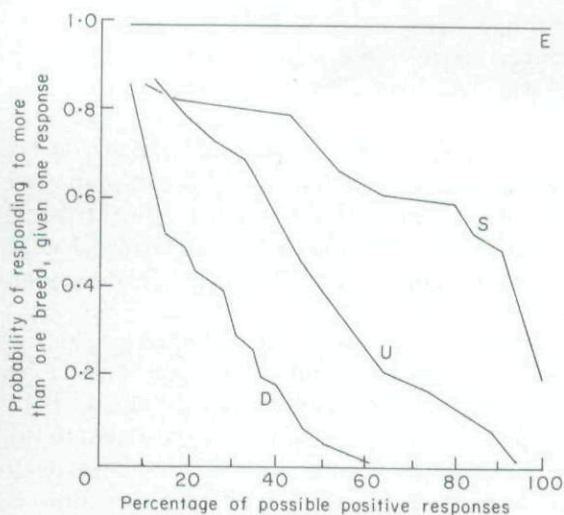


Fig. 1. Graphic representation of estimated probability distribution of responses to canine antigen type in the studied population. E=Expected response curve with no selective antigenicity; S=serum; D=dander; U=urine.

A variability in antigenic specificity between veterinarians and atopic patient responders is shown in Fig. 2, by recomputing the curves for the veterinarians and the atopics separately. In the case of each allergen, the curve for the atopic patient responders lies above the corresponding curve for the veterinarian responders ($P=0.001$). Skin test responses to a single antigen type ranged from 0 to 4+ in the same subjects.

Four veterinarians and two atopic patients responded strongly (3 to 4+) to a number of breed-specific sera and not at all to other breed-specific sera, suggesting

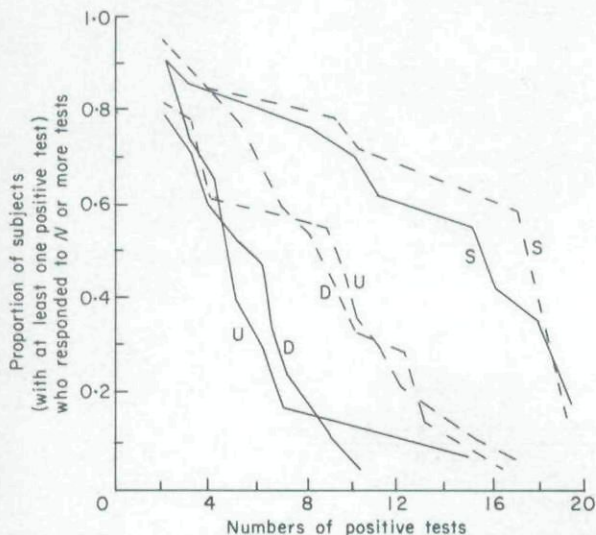


Fig. 2. Graphic representation of variability in reactions between skin responsive atopic patients (----) and veterinarians (—), using different canine antigen types. S=serum; D=dander; U=urine.

possible selective though variable sensitivity based on breed. Two of these six subjects had skin reactions to sixteen of seventeen breed-specific sera. The sera to which they did not react could not be considered weakly antigenic, since they evoked strong skin reactions in other tested subjects.

The results with urine antigens were striking. Twenty of twenty-five tested subjects with skin reactions to some breed-specific urine antigens and not to others also showed a pattern of selective responses with breed-specific serum antigens. Furthermore, subjects with strongly positive skin reactions to breed-specific dander extracts had at least one negative reaction to a breed-specific dander antigen extract, indicating some degree of selectivity in the responsiveness of those subjects.

Skin test findings from two dog-sensitive atopic patients are presented graphically in Fig. 3. A fairly high degree of 'selective sensitivity' or specificity was manifested by the differences seen in twenty-one of thirty-two (66%) skin test readings. In both patients, stronger reactions (4+) were seen to pure-bred dander extracts than to the mixed-dog dander screen. One of the atopic patients had positive skin responses to all of the breed-specific sera, while the other patient had no response. Non-atopic controls had no demonstrable skin responses to any of the antigens.

Discussion

Although criticized as a diagnostic technique by a number of authors (Norman, Lichtenstein & Ishizaka, 1973), skin testing continues to be the most widely utilized method in clinical use for detecting hypersensitivity. The criticism may be justified if appropriate antigens are not utilized to detect clinical sensitivity. In our test population, the mixed-dog commercially prepared dander extract did not appear to be an effective determinant of hypersensitivity to dogs. The results of testing with the commercial extract correlated poorly with the clinical history of hypersensitivity in dogs. In the present study, skin tests with pure-bred canine antigens evoked positive responses in almost 50% of the test subjects who had no response to the commercial

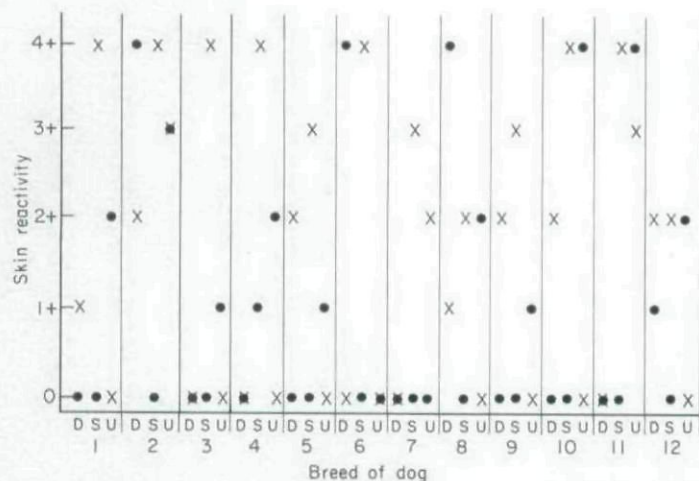


Fig. 3. Scattergram of skin reactions of two atopic patients using canine antigens of different types. Both patients (x, •) were reactive to a commercially mixed canine screen antigen. 1-12, different dog breed; D, dander from that breed; S, serum from that breed; U, urine from that breed.

screen. Currently available commercial mixed-dog danders or mixed-dog epithelial extracts give no indication as to type, breed or strain of dogs utilized in the preparation of the antigenic mixtures. These products usually represent the combined epithelial tissues of several mongrel (non pure-bred) dogs, with wide batch-to-batch variation (Greer Laboratories, personal communication). The results of the present study suggest that the spectrum of available dander extracts should be augmented to include specific breeds of dogs.

Rodent urine has been reported as causing hypersensitivity lung disease in exposed laboratory animal workers (Carroll *et al.*, 1975). More recently, cases of respiratory allergy of the immediate type were described in several laboratory workers (Taylor, Longbottom & Pepys, 1977). These individuals had asthma and other allergic manifestations and had IgE reactions as detected by prick and RAST test to urine proteins. The allergenicity of dog urine has not been studied heretofore, but sensitivity to canine urinary proteins can be presumed to develop from direct contact as well as the inhalation of dried urine particles. Dog serum albumin has previously been postulated as an allergen (Varga & Ceska, 1970). Our studies with pure-bred canine urine and serum indicate that both are potentially useful antigens for skin testing. Used in combination, these antigens are capable of identifying more positive skin test responders than either of them used singly. The highest detection rate of positive responders (98%) was seen when both serum and urine tests were made.

The addition of canine serum or urine antigens to dog dander extracts could prove to be a useful antigenic augmentation and provide a more accurate detection rate of individuals sensitive to dogs. In terms of reactivity, serum appears more reactive than dander, i.e., animal-sensitive individuals are more likely to react to serum than dander. This may be due to the increased protein content in serum, however, those reacting positively to serum are more likely to have reactions to more than one source of antigens, i.e., dander and urine.

The varied occupational animal exposure sustained by veterinarians in small animal practice may have enhanced their chances of developing increased sensitivity to antigens differing from those affecting the group of clinical atopics.

The variability in antigenic reactivity, as seen in Fig. 2, suggests that the atopic patients with positive skin tests reflect a lesser selectivity in their sensitivities, particularly to dander and urine, than did the veterinarians who manifested broad reactivity. Significant specific sensitization may thus occur. The disparity of responses with these pure-bred canine antigens suggests a specificity of skin reactivity based on breed of dog, or may be a variability in antigen preparation. If there were no specificity in response to different breeds, subjects would have positive responses to all antigens and the three response curves in Fig. 1 would be a straight line. The response curves are clearly different and thus indicate that reactivity to one breed of dog does not necessarily indicate a reactivity to all breeds.

There is some indication from this study that selective reactivity to dogs may occur. Variable responders, i.e., those test subjects showing a wide range (0 to 4+) of responses, represent indicators of selectivity. Our findings indicate that serum is more generally antigenic than urine or dander, but this may be related to increased antigenic protein in serum than in urine or dander.

Qualitative differences among canine danders, based on breed, were proposed by Hooker (1944). A variation in skin test responses of dog-sensitive patients suggests that there may exist major common as well as minor specific determinants among canine

dander allergens from different breeds (Fagerberg & Wide, 1970; Varga & Ceska, 1970). Our results with breed-specific serum and urine antigens, in addition to the dander antigens, indicate that cases of highly significant specific sensitization do occur. The disparity of responses with these purebred canine antigens indicates a specificity in skin reactivity based on breeds of dogs.

Thus, the results of this study suggest that the use of additional canine antigens such as urine and serum in addition to the conventional danders should be considered in testing for skin reactivity. Furthermore, the variability of sensitivity related to breed-specific antigens suggests that antigens from a wide variety of pure-bred dogs should be included in canine antigen preparations, and that these antigens may be of value in patients with histories of specific breed-associated symptoms. Our studies also indicate that much needs to be done in further characterization and standardization of canine antigens.

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