

Epithelial or dandruff allergen for the diagnosis of dog allergy by RAST?

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Summary

When used for screening by the RAST technique, dog epithelium provided too few positive results (29.2%) compared with dog dandruff (58.8%), cat epithelium (53.6%) and horse dandruff (52.2%). However, dog dandruff can provide an unexpectedly high incidence of positive RAST results (45.2%) in sera from patients with an allergic history to cat or horse, but not to dog. Dog dandruff allergens were found to cross-react with some cat or horse serum proteins in about 25% of such patients.

We conclude that dog dandruff is preferable to epithelium for screening by the RAST technique, but if emotional issues involving a family pet arise then confirmation by a trial separation is likely to be more convincing than a provocation test with a possibly non-specific allergenic extract.

Introduction

Two major allergens of dog-dandruff were found in dog serum; one was clearly serum albumin and the other appeared to be γ -globulin (Yman, Brandt & Ponterius, 1973). The same authors showed that albumin was also a major component of dog epithelium, and suggested that the γ -globulin component was also present in epithelial extracts but in too weak a concentration to be measured by the radioallergosorbent test (RAST)—(Wide, Bennich & Johansson, 1967). They suggested that differences in the strength of epithelial extracts taken from different breeds of dog may account for results indicating that some patients have breed-specific allergies (Hooker, 1944; Fagerberg & Wide, 1970). We have tested the practical importance of these findings by using dog epithelial and dandruff extracts for the estimation of IgE antibodies in serum from patients with a known history of hypersensitivity to dogs and to other animals.

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Methods

Patients

Sera from 121 patients with a known hypersensitivity to dogs, cats or horses were used for this study. Eighteen patients gave an allergic history to dogs only, fifty to dogs and either to cats or horses, but the remaining fifty-three were history-positive only to cat or horse. This patient material is grouped and summarized in Table 1. We used two groups of control sera: twenty-five from patients with a negative allergic history to animals but positive to *Dermatophagoides pteronyssinus*, and twenty-five from patients who were negative by RAST to a panel of nineteen U.K. allergens.

IgE tests

Total serum IgE was measured by a fast double-antibody technique (Merrett & Merrett, 1978), specific IgE measurements used Phadebas RAST reagents (Pharmacia GB Ltd., London), and a modification of the manufacturers' recommended procedure (John & Merrett, 1979). The commercial scoring system of zero, 1, 2, 3 and 4 was used, with zero regarded as negative, 1 as unlikely to be clinically significant, and with 2, 3 and 4 as increasingly positive.

RAST inhibitions involved an overnight incubation of human test serum (50 μ l) with an equal volume of the animal serum, albumin or γ -globulin fraction under test prior to the usual RAST procedure. The inhibiting serum was used neat, and as a series of five-fold dilutions.

The following animal sera were used: cat (Nordic Immunologicals Ltd, Maidenhead), dog and horse (Gibco Biocult Ltd, Hounslow); crude γ -globulin fractions of dog serum were prepared by precipitation at, and washing with, 18% sodium sulphate. Each mg of precipitate contained 40 μ g of albumin. Cat, dog and horse albumins (Sigma Chemical Co. Ltd, Poole) were used without any further purification.

Immunodiffusion

Double diffusion was performed by the method of Ouchterlony (1949), except that 2% agarose was substituted for agar. Sheep anti-dog serum was purchased from Nordic Immunologicals Ltd (Maidenhead).

Table 1. Numbers of patients listed according to a positive allergic history

	Number of patients with allergic history to			Number of patients per group
	Dog	Cat	Horse	
Group 1	18	0	0	18
Group 2	50	48	15	50
Group 3	0	49	8	53

Group 1, dogs; group 2, dogs, and cats and/or horses;
group 3, cats and/or horses.

Results

Correlation of RAST results with history

The agreement between positive RAST results and a positive history of animal allergy is shown in Fig. 1. When the 121 patients were grouped according to 188 specific histories, similar results were found (Table 2); thus, positive RAST results to dog dandruff, cat epithelium and horse dandruff generally occurred in 50%–60% of these patients – about twice the percentage recorded for dog epithelium.

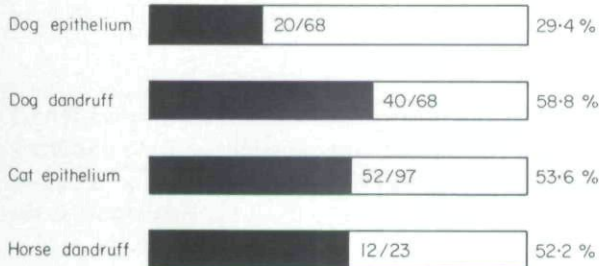


Fig. 1. Proportion of patients with a positive history of allergy to animals who also had a positive RAST result; e.g. of sixty-eight patients with a history of dog allergy, twenty (29.4%) gave a positive RAST result to dog epithelium.

Sera from patients who admitted no history of allergy to some animals were tested for specific IgE antibodies against extracts from these same animals. It was found (Fig. 2) that dog epithelium gave the largest proportion of negative results (83%) followed by horse dandruff (73.5%), cat epithelium (66.7%) and dog dandruff (54.8%). In other words, dog dandruff produced a much higher incidence of possibly false-positive RAST results than did dog epithelium. There was a similar incidence of positive RAST results in each group of patients examined, except for cat epithelium in group two (100%) and group three (zero %), but the numbers of patients involved were two and four respectively and, therefore, unlikely to be representative (Table 3).

Table 2. Incidence of positive RAST results in patients with the various animal allergies summarized in Table 1. Briefly, group 1 represents patients with positive histories to dogs only; group 2 positive to dogs and other(s); group 3 positive to cat and/or horse

RAST	Allergens	Percentage of patients with positive RAST results in			
		Group 1	Group 2	Group 3	All groups
Dog	epithelium	33.3	28.0	—	29.4
Dog	dandruff	61.1	58.0	—	58.8
Cat	epithelium	—	47.9	59.2	53.6
Horse	dandruff	—	53.3	50.0	52.2

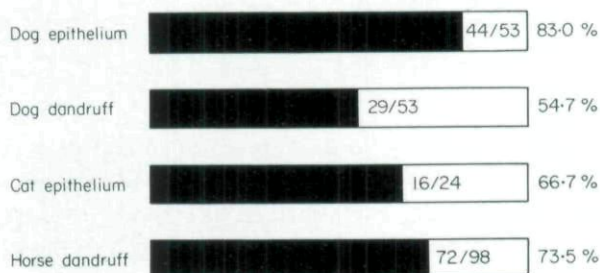


Fig. 2. Proportion of patients with no admitted history of allergy to certain animals who also had a negative RAST result; e.g. of 53 patients with no history of dog allergy 44 (83%) gave a negative RAST result to dog epithelium.

In the patients with no history of animal allergy, there were two positive RAST results to dog dandruff, but none to dog epithelium, cat epithelium or horse dandruff. The incidence of these positive dog dandruff results was 1/25 (4%) in the group of patients allergic only to house-dust mite, and 1/25 (4%) in the normal—that is, the non-atopic group.

Table 3. Incidence of positive RAST results in patients without positive histories of animal allergy (see Table 1 for details of patient material)

RAST allergens	Percentage of patients with positive RAST results in			
	Group 1	Group 2	Group 3	All groups
Dog epithelium	—	—	17.0	17.0
Dog dandruff	—	—	45.2	45.2
Cat epithelium	33.3	100	0	33.3
Horse dandruff	27.8	28.6	24.4	26.5

RAST inhibition by animal sera

Inhibition of RAST by homologous sera was variable. Thus, in serum from one patient allergic to cat, dog and horse, the positive dog and horse RAST results could be inhibited with dog and horse sera respectively, but cat serum did not inhibit the positive cat RAST (Fig. 3). We noted that dog serum was more likely to inhibit dog dandruff RAST in patients with a history of dog allergy (12/19) than in those without (6/16).

Inhibition of dog dandruff RAST by heterologous sera was also variable, but less common than by dog serum. Thus, when the same thirty-five patients' sera were tested, we found that three were inhibited by cat serum and another six by horse serum.

Figure 4 shows that in eighteen sera RAST-positive to dog dandruff but negative to dog epithelium, the crude dog γ -globulin fraction—prepared by sodium sulphate precipitation—usually inhibited dog dandruff RAST more than a similar concentration of dog albumin; this was always true if the patient admitted to a history of dog allergy.

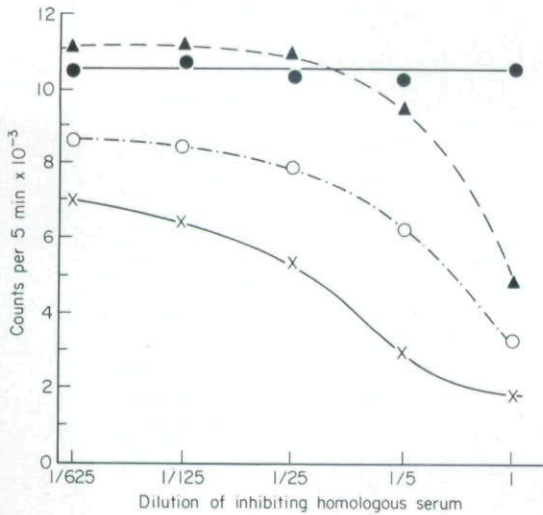


Fig. 3. Inhibition of one patient's RAST results with homologous animal serum; i.e. different dilutions of (a) cat serum is used to inhibit cat RAST, (b) dog serum to inhibit dog RASTs, and (c) horse serum to inhibit horse RAST. ●, Cat epithelium; ▲, dog dandruff; ○, dog epithelium; ×, horse dandruff.

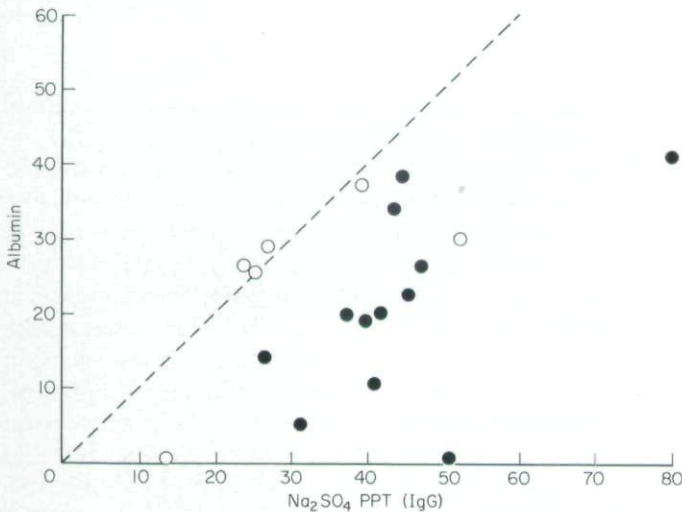


Fig. 4. Correlation between the percentage inhibition of dog dandruff RAST by 2.5 mg of dog albumin and 2.5 mg of crude dog γ -globulin (prepared by precipitation with 18% sodium sulphate). Out of eighteen patients, twelve gave a positive history of dog allergy (●) and six did not (○).

Immunodiffusion

On double diffusion in agarose sheep anti-dog serum precipitated with cat, dog, horse and human sera (Fig. 5); horse and human sera were the only ones which clearly showed a line of identity.

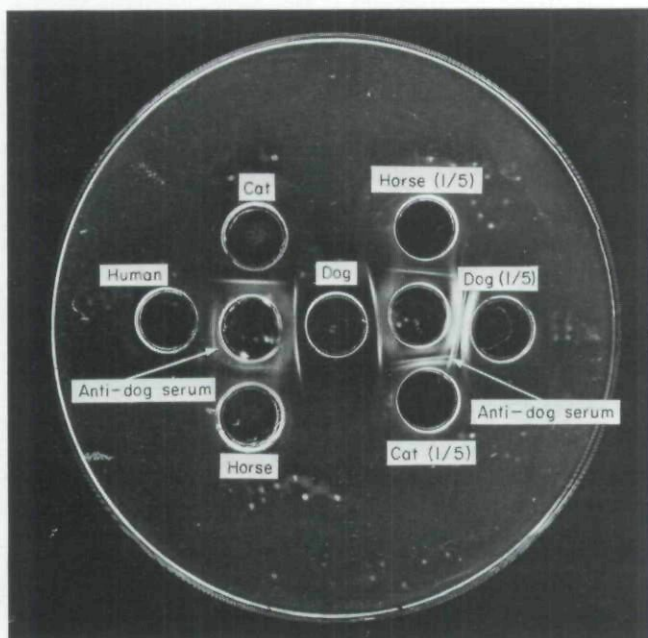


Fig. 5. Immunodiffusion analyses of cat, dog, horse and human sera against sheep anti-dog serum.

Discussion

The object of this study was to decide whether dog dandruff or dog epithelium should be used in the RAST techniques for diagnosing IgE-mediated dog allergies.

Agreement between RAST and a positive case-history was about 55% for cat and horse allergens and also for dog dandruff, but for dog epithelium it was only about 30% (Fig. 1). These correlations compared unfavourably with a reported 89% in a study of horse allergies (Leegaard & Roth, 1977), but were similar to a 55% incidence of a positive RAST result for cat allergen (a pre-albumin) in fifty-eight patients with a positive history of allergy to cats (Ohman, Lowell & Bloch, 1974). These lower results might be due to our patients either having successfully avoided the animal in question or not having IgE-mediated allergies – although all, except three, had total serum IgE levels over 40 u/ml. Whatever the reason, we found that dog dandruff was as successful as cat epithelium and horse dandruff for screening by the RAST technique, but that dog epithelium is at present unsuitable. Kleinhans (1977) also reported that a RAST technique was unsuitable for screening dog epithelium. RAST inhibitions with albumin and a crude γ -globulin fraction of dog serum (Fig. 4) suggested, as did the work of Yman and his colleagues (1973), that this was likely to be due to an insufficiency of γ -globulin in epithelial extracts.

Nonetheless, dog dandruff was not entirely satisfactory, since it provided apparently 'false-positive' results in 45.2% of patients with a history of allergy to either cats or horses but not to dogs (Fig. 2). This suggested that there are cross-reacting allergens between these species, and we found that out of thirty-five patients' sera positive by RAST to dog dandruff nine (25.7%) could be inhibited by cat or horse serum. Furthermore, anti-dog serum recognized components present in cat, horse and human

serum (Fig. 5). However, a proportion a 'false-positive' results might be accounted for if misleading answers were given to questions relating symptoms to family pets. Warner (1977) stated that emotional issues involving family pets are best resolved by *in-vivo* provocation testing, but the possibility of cross-reacting allergens from different species suggests that a trial removal of the pet following a positive screening test (either RAST or skin-prick test) is more likely to provide an unambiguous result.

If it is assumed that most animal skin extracts contain a variety of allergens, and that each allergen has more than one determinant, then each person with an animal allergy is likely to have circulating a spectrum of animal-specific IgE antibodies. This spectrum will vary between patients, and a multi-allergen extract is more useful for screening than a single pure allergen. Therefore, we conclude that for a screening test by RAST it is better to use dog dandruff than dog epithelium.

Acknowledgments

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References

- FAGERBERG, E. & WIDE, L. (1970) Diagnosis of hypersensitivity to dog epithelium in patients with asthma bronchiale. *International Archives of Allergy and Applied Immunology*, **39**, 301.
- HOOKE, S.B. (1944) Qualitative differences among canine danders. *Annals of Allergy*, **2**, 281.
- JOHN, A.C. & MERRETT, T.G. (1979) The Radioallergosorbent test in polyposis. *Journal of Laryngology and Otology*. In the press.
- KLEINHANS, D. (1977) Allergenspezifisches immunoglobulin E in serum (RAST) bei Tiererepithelien-sensibilisierung. *Medizinische Welt*, **28**, 630.
- LEEGAARD, J. & ROTH, A. (1977) RAST in the diagnosis of hypersensitivity to horse allergens. A comparison with clinical history and *in-vivo* tests. *Clinical Allergy*, **7**, 455.
- MERRETT, T.G. & MERRETT, J. (1978) Methods of quantifying circulating IgE. *Clinical Allergy*, **8**, 543.
- OHMAN, J.L. JR, LOWELL, F.C. & BLOCH, K.J. (1974) Allergens of mammalian origin. III. Properties of a major feline allergen. *Journal of Immunology*, **113**, 1668.
- OUCHTERLONY, O. (1949) *In-vitro* method for testing toxin-producing capacity of diphtheria bacteria. *Acta Pathologica Microbiologica Scandinavica*, **26**, 516.
- WARNER, J.O. (1977) Bronchial provocation tests. *Archives of Disease in Childhood*, **52**, 750.
- WIDE, L., BENNICH, H. & JOHANSSON, S.G.O. (1967) Diagnosis of allergy by an *in-vitro* test for allergen antibodies. *Lancet*, **ii**, 1105.
- YMAN, L., BRANDT, R. & PONTERIUS, G. (1973) Serum albumin - an important allergen in dog epithelia extracts. *International Archives of Allergy and Applied Immunology*, **44**, 358.
- YMAN, L., PONTERIUS, G. & BRANDT, R. (1975) RAST-based allergen assay methods. In: *Developments in Biological Standardization, Standardization and Controls of Allergens Administered to Man*. Volume 29, Karger, Basel.

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