

Cross-allergenicity between extracts of hair from different dog breeds and cat fur

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Summary

Skin tests and RAST determinations with breed-specific dog allergen extracts and a cat allergen preparation were made on forty-four atopic patients divided into three groups.

Group 1 were twenty dog-owning atopic patients without clinical signs of dog sensitivity, Group 2 contained twenty-one patients with a clinical history that suggested allergy to dogs, and Group 3 contained ten atopic patients who were sensitive to cats. In neither the *in vivo* nor the *in vitro* tests was there any evidence for dog breed specificity, nor was dog albumin found to be a major allergen in the population studied, though a few individuals showed strong RAST activity to albumin. Furthermore, a cat fur extract inhibited the reaction between dog hair and anti-dog serum, and a dog hair extract inhibited the reaction between cat fur extract and anti-cat serum.

Introduction

Hypersensitivity to animal antigens has long been recognized (Dunbar, 1913) and allergic disease of the airways is common in pet owners (Schultze-Werninghaus, 1981). Clinical hypersensitivity is said to occur in 40-60% of people with long exposure to guinea-pigs and hamsters, whereas sensitivity to cats and dogs may occur in 20-40% exposed atopic persons (Lutsky & Neuman, 1975; Schultz-Werninghaus *et al.*, 1976; Rudolph *et al.*, 1981). Breed-specific allergy to dogs has been described (Hooker, 1944; Voorhorst, 1965; Fagerberg & Wide, 1970; Moore, Hyde & Manaligod, 1977; Moore & Hyde, 1980), evidence of the existence of common major dog allergens has also been presented (Blands, Lowenstein & Weeke, 1977, McLean *et al.*, 1980, Koers, Young & Berrens, 1977, Brandt & Yman, 1980). In a previous study designed to find the most useful dog allergen preparation for RAST to detect specific IgE in patients with suspected allergy to dog and not cross-reacting with cat antigens, we concluded that

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none of the preparations tested was ideal for a good correlation with high specificity in *in vivo* tests and in RAST (Wüthrich & Arrendal, 1979). In contradiction with other investigators (Yman, Blomberg & Schroder, 1973) we also found that none of the dog-sensitive patients could be identified by RAST using dog serum discs. The present work is an investigation of skin sensitivity and specific serum IgE assays in forty-four individuals who were in contact with dogs, to try to decide whether breed specificity exists and to examine again the importance of dog albumin in patients who are allergic to dogs.

Materials and methods

Patients

Forty-four out-patients with perennial respiratory symptoms and with exposure to dog and/or cat were studied. None had been hyposensitized to either dog or cat.

Group 1 (patients 1–20). Twenty dog-owning atopic patients who were not considered to be sensitive to either dogs or cats from the evidence of: (i) clinical history; (ii) negative intracutaneous tests with commercial mixed dog hair and cut fur extracts (Stallergènes test concentration 10^{-3} w/v; and (iii) negative RAST results using commercial dog and cat epithelium discs (Phadebas RAST, Pharmacia, cat e1, dog e2).

Group 2 (patients 21–42). Twenty-two patients for whom clinical history and skin tests or RAST were suggestive of specific allergy to dogs.

Group 3 (patients 34–44). Eleven patients who from clinical history and pretesting by RAST using cat allergen discs (Pharmacia) and/or intracutaneous tests with Stallergènes cat fur extract were considered sensitive to cats (patients 34–42 were thus sensitive to both dogs and cats).

Skin tests

Patients were prick tested using 10^{-3} and 10^{-2} w/v solutions and also tested intracutaneously (0.02–0.03 ml) using 10^{-6} – 10^{-3} w/v solutions.

Allergen extract 1. Commercial mixed dog hair extract (Stallergènes). Equal parts of hair clipped from five breeds of dog were mixed and the soluble allergens extracted at the rate of 10 g hair to 100 ml 50% glycerol saline solution (pH 6.0).

Allergen extract 2. Commercial fur extract (Stallergènes). Fur clipped from cats was mixed and the soluble allergens extracted as in extract 1.

Allergen extract 3. Specific dog breed hair extracts. Hair clipped from individual animals of the same breed was combined and the soluble allergens extracted as in extract 1. The protein concentration of the breed-specific extracts were determined by Lowry *et al.* (1951).

Poodle:	0.72 mg/ml.
Beagle:	1.60 mg/ml.
Dachshund:	1.90 mg/ml.
German shepherd:	0.75 mg/ml.
Boxer:	1.15 mg/ml.

Allergen extract 4. Dog albumin. A 10% solution prepared from Albu-dog (Merieux Ltd) was used for *in vitro* determinations only.

In vitro tests

Blood was drawn at the time of skin testing and all sera were tested in Zurich for

specific IgE by Phadebas RAST against commercial dog and cat epithelium discs (Pharmacia). Sera were frozen and stored at -30°C and then tested under code against specific dog hair extracts and dog albumin.

Direct RAST

Breed-specific paper discs were prepared using $50\ \mu\text{l}$ of a 10^{-1} w/v glycerol saline extract per cyanogen-bromide-activated disc. Uncoupled sites were blocked using EPA 33. In the RAST, $50\ \mu\text{l}$ of each patient's serum diluted 1:5 v/v in phosphate-buffered saline (PBS) were incubated at room temperature for 3 hr with duplicate discs of each insolubilized allergen. Bound IgE was revealed using ^{125}I -labelled rabbit anti-human IgE (Pharmacia Ltd). Results were reported as the ratio of the cpm given by the serum compared to the cpm given by a 0.1% human serum albumin solution.

A ratio ≥ 1.7 was considered positive (Wüthrich *et al.*, 1981).

RAST inhibition

This was done by the method devised by the IABS Working Party for Standardization of the RAST (1977). Briefly, serial dilutions of inhibiting extract were added to $100\ \mu\text{l}$ of serum pool and incubated for 1 hr at room temperature. An allergen disc sensitized with the appropriate allergen for the serum was added to each mixture and the RAST was completed as described above.

To show efficacy of the system, a first titration was done in which aliquots of a pool of serum from dog-sensitive patients was first inhibited with mixed dog extract, then the mixtures were applied to discs that had been sensitized with mixed dog extract. This was the 'dog-on-dog' inhibition system.

Subsequently the same serum was inhibited with cat extract instead of mixed dog, and the assay made using mixed dog discs as before. This was the 'cat-on-dog' inhibition system.

Then aliquots of an anti-cat serum pool were inhibited with cat extract and the mixtures assayed on cat sensitized discs. This was the 'cat-on-cat' inhibition system.

Finally the anti-cat serum pool aliquots were inhibited with mixed dog extract, and the assay done with cat-sensitized discs as before. This was the 'dog-on-cat' inhibition system.

Results

Group 1: clinically not allergic to dogs

Although three of these patients showed slightly raised IgE titres to some breeds and there were some weak positive intracutaneous reactions at 10^{-3} and 10^{-4} allergen extract concentration, especially against poodle, mixed dog and dachshund extracts, only one had a positive prick test to poodle. None of these patients showed clinical symptoms of allergy when exposed to dogs. It must be concluded that neither of these tests are absolutely diagnostic of clinical hypersensitivity and that some people living in close proximity with dogs, develop low levels of IgE antibody, which, whilst it may be skin sensitizing at a low level of activity, does not mediate symptoms of allergic disease. Cross-reactivity with other atopic allergens must also be taken into consideration.

Group 2: clinically allergic to dogs

All patients allergic to dogs had positive intracutaneous tests at least at 10^{-3} allergen

concentration with all the dog extracts but the Pharmacia RAST was negative for three patients. The RAST using individual breed extracts was strongly positive for one patient however although two other patients were RAST-positive only with the dachshund extract. As the RAST responses to different dog breed extracts showed some variability, data from this group were analysed by Student's *t*-test to assess whether there was any significant difference in response to the different breed extracts. The activity of all the extracts was compared with that of the poodle. The results in Table 1 show that this group of patients showed no significant difference in response to the five specific extracts but that the response to dog albumin was significantly different ($P < 0.05$).

Group 3: patients clinically allergic to cats and/or to dogs

Five of these patients showed very high humoral IgE titres to the dog breed-specific extracts (RAST titre > 5) and had intracutaneous reactions at high allergen dilution even though patients only possessed one breed of dog. All patients, with one exception, also gave a positive intracutaneous reaction to cats. Two patients were allergic to cats only and showed no specific IgE to any of the dog breeds.

RAST inhibition results

'Dog-on-dog' system (Fig. 1). The inhibition titration is shown in Fig. 1. The calculated 50% inhibition end-point was $0.69 \mu\text{l}$.

'Cat-on-dog' system (Fig. 1). When cat extract was added to the dog extract-dog serum system, significant inhibition was seen. The calculated 50% inhibition end-point was $3.43 \mu\text{l}$, about 5 times that needed for the homologous dog extract, but still a significantly small amount of cat extract.

'Cat-on-cat' system (Fig. 2). Here the inhibition titration is also good, with a 50% inhibition end-point of $0.42 \mu\text{l}$, indicating a potent cat hair extract.

'Dog-on-cat' system (Fig. 2). The effect of dog extract on the cat extract-cat serum system gives a 50% inhibition end-point of $78.63 \mu\text{l}$. This is a smaller effect which is still significant.

Table 1. Mean responses to five different extracts (Group 2)

Extract	Mean Response	s.d.	s.e.m.	F		TDL = 42	Significant $P < 0.05$
				Calculated	Theoretical		
Poodle	2296.91	17775.02	378.44	—	6	—	—
Beagle	2433.73	1992.06	424.71	1.59	2.10	0.24	n.s.
Dachshund	2807.36	1968.77	419.74	1.51	2.10	0.90	n.s.
German shepherd	2320.55	1765.15	376.33	1.02	2.10	0.04	n.s.
Boxer	2422.09	1909.15	407.03	1.34	2.10	0.23	n.s.
Dog albumin	940.55	1470.04	313.41	2.13	2.10	2.76	s.

Discussion

In neither the *in vitro* nor *in vivo* tests have we been able to show breed specificity between tests with extracts from different breeds of dog. Such variations in RAST titres

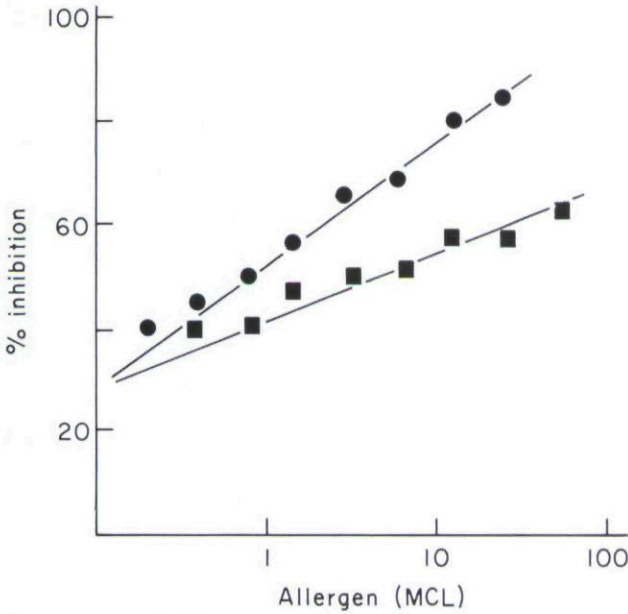


Fig. 1. RAST inhibition titration. Comparison of dog allergen with cat allergen to inhibit anti-dog IgE reacting with disc with dog allergen.

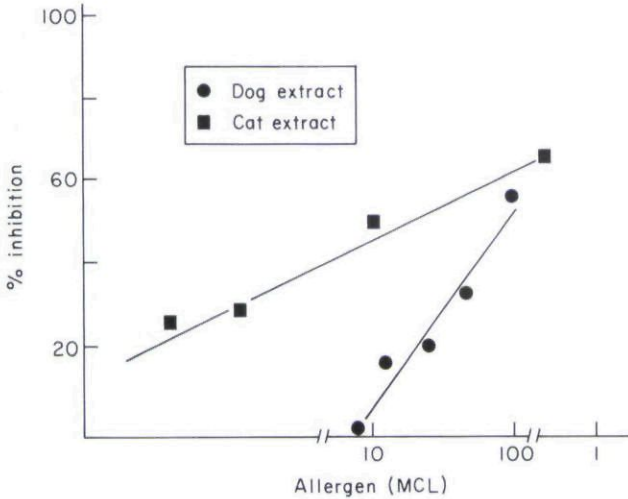


Fig. 2. RAST inhibition titration. Comparison of dog allergen with cat allergen to inhibit anti-cat IgE reacting with disc with cat allergen.

between breeds that we saw were small and are most probably due to experimental variation.

Although five of the patients gave a weak intracutaneous reaction with the poodle extract when none was seen with the mixed breed extract that is usually used for skin tests, we do not believe that this constitutes evidence for breed specificity. It is more likely to be a concentration of active allergen effect, although the poodle extract contained less PN than any other extract. There is no evidence for improved diagnosis

using breed-specific allergens rather than the routine mixed breed extract, since all of these five patients were in Group 1, where clinical history indicated no sensitivity to dogs.

Those patients who had very high IgE titres all fell into Groups 2 and 3, where the limiting dilution for intracutaneous tests was also high, and the patients gave a clinical history of disease caused by contact with their dogs.

It is interesting that eighteen of the forty-four patients also reacted to extract of cat fur. There could be two reasons for this: (i) there could be a cross-reacting allergen in cat and dog hair, or (ii) those who form IgE specific to dog allergens also make separate IgE specific for cat, simultaneously.

The RAST cross-inhibition *in vitro* studies clearly show that a component of the cat extract is able to interfere in the reaction between dog extract and dog serum and this indicates substantial common allergenicity of the dog and cat extracts, which is confirmed, though less clearly, by the effect of dog extract on the cat extract-cat serum system.

There were twelve patients in Group 1 who gave weak positive intracutaneous tests to dog hair, with no clinical history of sensitivity of these animals, and we must conclude that either these were false positives, or the skin hypersensitivity was not associated with clinical sensitivity.

Finally, although Yman *et al.* (1980) found dog serum albumin to be an important allergen in dog hair extracts, our *in vitro* RAST data shows this only for a few patients, whilst most of the sera that gave positive results with the dog hair extract discs gave negative results with discs charged with dog albumin. Since some sera gave very high titres with the dog albumin discs we conclude that they were immunologically active, and where a negative result was seen it seems that there was no antibody and we are obliged to conclude that, whilst some patients react strongly to dog albumin, most do not, and in the majority of cases the allergens from hair are of paramount importance (Hoffman, 1980; Merritt & Merritt, 1979; Vanto *et al.*, 1978).

References

- BLANDS, J., LOWENSTEIN, H. & WEEKE, B. (1977) Characterisation of extract of dog hair and dandruff from six different dog breeds by quantitative immunoelectrophoresis. Identification of allergens by crossed immunoelectrophoresis (CRIE). *Acta Allergologica*, **32**, 147.
- BRANDT, R. & YMAN, J.L. (1980) Dog dander allergens. Specificity studies based on the radioallergosorbent technique. *International Archives of Allergy and Applied Immunology*, **61**, 361.
- DUNBAR, W.P. (1913) The present state of our knowledge of hay fever. *Journal of Hygiene*, **13**(2), 106.
- 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.
- HOFFMAN, D.R. (1980) Dog and cat allergens: urinary proteins or dander proteins? *Annals of Allergy*, **45**, 205.
- HOOKE, S.B. (1944) Qualitative differences among canine dander. *Annals of Allergy*, **2**, 281.
- IABS WORKING PARTY FOR STANDARDISATION OF THE RAST (1977) *Standardisation and Control of Allergens Administered to Man*, **29**, 438.
- KOERS, W.J., YOUNG, E. & BERRENS, L. (1977) Specific and non-specific skin test reaction with animal dander extract. *Allergy and Immunology*, **23**, 261.
- LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.S. (1951) Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265.
- LUTSKY, I. & NEUMAN, I. (1975) Laboratory animal dander allergy. I. An occupational disease. *Annals of Allergy*, **35**, 201.
- MCLEAN, A.C., GLOVSKY, M.M., HOFFMAN, D.R. & GHEKIERE, L.M. (1980) Identification of allergens in

- dog dander extract. I. Clinical and immunological aspects of allergenicity activity. *Annals of Allergy*, **45**, 199.
- MERRETT, T.G. & MERRETT, J. (1979) Epithelial or dandruff allergen for the diagnosis of dog allergy by RAST? *Clinical Allergy*, **9**, 429.
- MOORE, L.S. & HYDE, J.J. (1981) Breed specific dog hypersensitivity in humans. *Journal of Allergy and Clinical Immunology*, **66**, 198.
- MOORE, B.S., HYDE, J.J. & MANALIGOD, L.M. (1977) A comparative study of allergens of canine origin. *Annals of Allergy*, **39**, 240.
- RUDOLPH, R., KUNKEL, G., BLOHN, B., MUCKELMANN, R., MAST, H. & KIRCHOF, E. (1981) Zur Häufigkeit und klinischen Bedeutung von Allergien gegen Tierepithelien. *Allergologie*, **4**, 230.
- SCHULTZE-WERNINGHAUS, G. (1981) Atemwegserkrankungen durch Soforttypallergie gegen Tierepithelien. *Allergologie*, **4**, 298.
- SCHULTZE-WERNINGHAUS, G., GONSIOR, E., THIEL, C., KROIDL, R. & MEIER-SYDOW, J. (1976) Häufigkeit und Korrelative Beziehung von diagnostischen kriterien bei der Tierschuppen-Allergie. *Acta Allergologica*, **31**, 44.
- VANTO, T., VIANDER, M., KOIVIKKO, A., SCHWARTZ, B. & LOWENSTEIN, H. (1982) RAST in the diagnosis of dog dander allergy. A comparison between three allergen preparations using two variants of RAST. *Allergy*, **37**, 75.
- VOORHORST, R. (1965) The dander allergens as a cause of atopic vasomotor rhinitis. *International Rhinology (Leiden)*, **3**, 65.
- WÜTHRICH, B. & ARRENDAL, H. (1979) RAST in the diagnosis of hypersensitivity to dog and cat allergens. A comparison of different extract preparations with clinical history, skin test and provocation tests. *Clinical Allergy*, **9**, 191.
- WÜTHRICH, B., GUERIN, B., HEWITT, B. & LUGGEN-BRUN, H. (1981) House dust allergy: correlation between *in vivo* and *in vitro* diagnostic tests. *Annals of Allergy*, **46**, 100.
- YMAN, L., BLOMBERG, F. & SCHRODER, H. (1980) Characterisation of dog and cat allergens. Direct specific detection of electrophoretically separated allergens by means of IgE antibodies and enzyme labelled anti-IgE. In: *Advances in Allergology and Immunology* (ed. Oehling *et al.*). Pergamon Press, Oxford.

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