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THE SPECIFICITY OF ANIMAL DANDER ALLERGENS: A SEMI-BLIND CLINICAL AND EXPERIMENTAL STUDY

By

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Apart from the allergens in the skin flakes of man and horses, little is known of the chemical nature and immunological specificity of the skin-reactive allergens of animal epithelial tissue in general (3). However, in the practice of allergy, there is a need of accurate information on vital issues such as allergen content of various animal dander extracts, standardization, species-specificity, cross-reactivities, etc.

Though neither allergen has been isolated in pure form, it has been shown that highly purified human and horse dander allergens most probably represent 2.5-3.4 S α -glycoproteins with isoelectric points of pI 4.1-4.2 (2, 17). In the human material, trace contamination of (antigenically modified) serum albumin and IgG was noted, together with a considerable proportion of 2.5 S serum α_2 -glycoproteins. The human allergen was demonstrated to occur in sweat collected from the skin surface (1).

In a recent series of experiments, *Ceska* and colleagues (10, 11, 18) have shown that the dander extracts of cat, dog, horse and cow, separated by gel isoelectrofocussing, contain common major components of pI 4.5-5.0. These bind IgE from the serum of sensitive individuals, as determined by radioallergo-sorbent test, using ^{125}I -labelled anti-(Fc) IgE(ND). However,

binding antigens of different pI were also noted, which were believed to combine with distinct IgE counterparts. Though no *in vivo* tests were performed, the conclusion of these authors, namely that there may exist major common as well as minor specific determinants among dander allergens from different (dog) breeds, is corroborated by the results of Fagerberg & Wide (12).

We have approached this problem by performing skin tests with different animal dander extracts prepared under standard conditions and submitted to the clinical investigators under code; simultaneously, a newly developed *in vitro* assay based on human complement consumption was employed (8, 9). A preliminary account of this work has been given (7).

MATERIALS AND METHODS

Allergens

Dander material was obtained from different groups of animals by carefully clipping the fur close to the skin with an electric razor. The samples were from Alaska female rabbits, female guinea pigs, female Swiss random-bred mice, male hamsters, female Wistar random-bred rats, the fur of one cat and hair from one (poodle) dog. Hairs were weighed and washed twice in acetone. The acetone phase was filtered through Whatman no. 1 filter paper. Powdered defatted dander remaining on the filters was air-dried, weighed, and extracted for one day at room temperature with 1 per cent NaCl-1 per cent phenol, then with 1 per cent NaCl, and finally with water. The combined extracts were dialysed against distilled water, and the nondialysable material was lyophilized. Yields were recorded, ultraviolet absorption and fluorescence emission, nitrogen and carbohydrate were determined as before (4).

Complement consumption

Complement consumption tests with *human* complement in fluid phase were performed as follows (9). Samples of 15 μ l of a patient's serum were incubated with varying amounts of dander allergen in a total volume of 0.5 ml Ca^{++} - Mg^{++} -veronal-saline buffer (VSB $^{++}$) made up according to Kabat & Mayer (13). Time of incubation: 30 min. at 37° C. Residual Ch^u was then assayed by adding 2.5 ml of VSB $^{++}$, followed by 1.0 ml of sheep red blood cells (SRBC) optimally sensitized with rabbit anti (SRBC) haemolytic antibody (A), 2.5×10^8 cells/ml. After 60 min at 37° C, residual Ch^u was evaluated by observing haemoglobin in supernatants at 541 nm (13). Complement consumption was expressed in terms of decreasing Z-values (13, 16), where $Z = -\ln (1-y)$ and y represents percentage haemolysis.

Skin tests

Intradermal or prick tests were performed by routine techniques in atopic patients, employing the allergen concentrations mentioned in the Results section. Patients were selected on the basis of their clinical symptoms, a family history of atopy, a clinical history of animal dander contact and/or sensitivity, manifested by positive weal and flare skin responses to the injection of commercial dander extracts. The majority of these patients displayed positive skin reactions to purified house dust and human dandruff allergen.

RESULTS

Recoveries and composition

It is common practice to adjust the strength of an allergenic extract on a weight (of dry substrate) per volume (of extracting fluid) basis. The allergen concentration per ml of extract then clearly depends on the allergen content of the dry dander, which may differ among danders of different sources. A first step towards better standardization might be based on the consideration that the common inhalant allergens possess molecular weights that do not allow them to pass through dialysis membranes (4). However, as shown in Fig. 1, the yield of total nondialysable substance—which should embrace all the allergens—as well as the proportion of skin scales adhering to hair varies considerably among the different animal danders. Hence, conventional weight per volume extracts contain variable amounts of high-molecular weight material. The highest proportion of potential allergenic substance was obtained from the dander of dog, horse, cat and rabbits. In some cases, for example with the Swiss randombred mice, the extremely low yields of soluble material hampered further analytical and isolation work. Even though it was recognized that much of the total nondialysable extract material must be non-allergenic components (e. g. mucopolysaccharides (4)), it was therefore decided to standardize test solutions for the present studies on the basis of equal *concentrations* of total nondialysable soluble (lyophilized) substance per ml of aqueous solvent.

Physicochemically, the dander extracts all featured the UV-

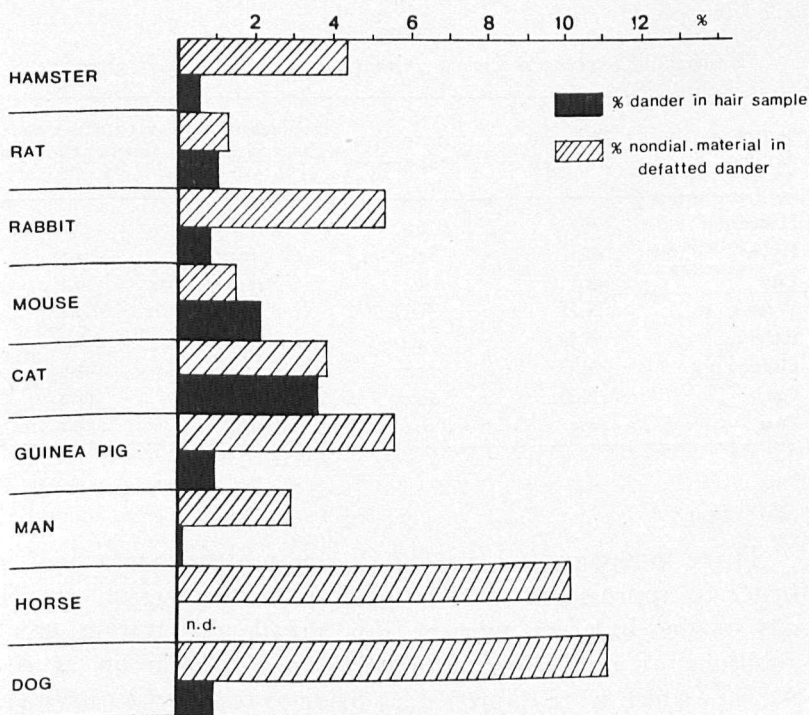


Fig. 1.

Diagram showing the percentage of dry dander obtained from the samples of hair used in this study, and the percentage of total extractable, nondialysable substance extractable from these danders.

absorption spectra considered typical of allergenic extracts (4). Some numerical data have been listed in Table 1.

The dander extracts, at 3 per cent concentration, were examined by electrophoresis in agar gel at pH 8.6, using carbohydrate and protein stains for visualization (2). Except for the known protein composition of the horse (17), human (2) and cow dander extracts (14, 15), no polysaccharides or proteins that could be stained in a conventional manner were noted in the extracts of the other dander extracts. Only the horse dander extract produced a faint precipitation line during immunoelectrophoretic analysis against rabbit antiserum to purified human dandruff allergen, as described before (2).

TABLE 1

Analysis of total nondialysable substance in different dander extracts.

Source of dander	Per cent Nitrogen	Per cent Total hexose	Extinction coefficient at 280 nm (1%, pH 7.0)	Extinction coefficient at 305 nm (1%, pH 7.0) *
Human	6.1	6.4	10.0	5.2
Horse	10.2	13.9	10.4	4.5
Cow	7.5	5.7	18.0	9.0
Wistar rat	N.D.	N.D.	5.4	3.4
Rabbit	N.D.	4.4	13.2	8.0
Guinea pig	N.D.	15.2	19.0	11.9
Cat	N.D.	12.7	16.7	11.2
Dog	8.3	10.2	20.4	13.2

Skin tests

Three independent series of skin tests were performed in order to appraise the potency and the allergological specificity of the different extracts. For intradermal testing, stock solutions of nondialysable material were made up at 0.5 mg/ml, which were diluted 1:25 prior to testing (20 µg/ml); for prick testing, the 0.5 mg/ml solution was used undiluted. All extracts were coded by alphabetical numbering, which was different for the three separate series. The code was broken after evaluation of the completed sets of skin test results.

TABLE 2

Results of intradermal tests with purified house dust allergen (fraction E, 2 µg/ml) and different dander extracts (20 µg/ml), first series.
Codes: C = horse dander; D = rabbit dander; E = guinea pig dander; F = cat dander.

	Number of patients	House dust positive; C, D, E, F positive	House dust negative; C, D, E, F positive	Negative controls
Total	26	18	4	4
Atopic	21	17	4	0
Non-atopic	5	1	0	4

TABLE 3

Skin-reactivities of soluble dander proteins, in units/mg, averaged over the total group of patients in Table 2, and prediction of potencies in classical (w/v) extracts.*

Source of dander	Nondial. soluble protein (mg/g dander)	Skin-reactivity found (units/mg)	Allergenic activity in units/ml predicted in 0.1 "percent" conventional extracts
Horse	100	113.400	11.340
Cat	38	13.100	498
Rabbit	53	10.250	543
Guinea pig	55	1.500	82

* 1 unit of allergenic activity is defined as the amount of lyophilized material (in mg) causing a 1+ positive skin reaction on average in a group of atopic subjects; hence, the activities filed here represent the reciprocal of the quantity of substance, in mg, required for a 1+ skin reaction (weal, no more than 5 mm diameter with associated erythema). (4)

a) *First series*

The number of patients involved, and the overall results of the tests have been collected in Table 2.

From an analysis of the skin test results and the history in individual patients, we were unable at the time (7) to decide whether or not the dander extracts had diagnostic value in detecting *specific* sensitization or whether the dander extracts were species-specific. Evaluation of the clinical data revealed several atopic patients who had not been suspected of animal dander sensitivities, yet reacted to the intracutaneous allergens in an irregular fashion (most often to the horse dander allergen). For example, one patient—positive to house dust—reacted to horse and cat dander, despite the absence of animal contacts. Conversely, a patient was investigated who did not react to house dust, had a history of asthma and urticaria by horse dander, and presented impressive skin reactions to human, horse, rabbit, cat and guinea pig dander.

Disregarding specificities, the mean allergenic potency of the dander extracts were calculated per mg of soluble sub-

stance on the basis of the overall positive tests, and expressed in units/mg as reported before (4). This permits an appraisal of the relative potencies of the dander extracts and—in combination with the recovery data of Fig. 1—allows of a prediction of the test strength of conventionally prepared (w/v) extracts. The results are shown in Table 3.

b) *Second series*

For the investigation of a second series of patients, fresh solutions were prepared, and the codes were changed. Prick tests were performed in 11 patients, at standard concentrations of 0.5 mg/ml. The results are shown collectively in Table 4.

TABLE 4

Results of prick-tests with house dust and different (coded) dander allergens in a second group of patients. Test concentrations: 0.5 mg/ml. A = cow; B = horse; D = dog; E = guinea pig; F = cat; G = rabbit dander; the + sign indicates a positive skin reaction, disregarding the gradation.

Patient	A	B	D	E	F	G	House dust	Remarks
1	o	o	o	o	+	o	+	
2	o	+	+	o	+	o	+	Clinically, sensitive to horse, dog and cat dander.
3	o	+	+	o	+	o	+	Previous i.c. tests positive to horse dander only.
4	o	o	+	o	+	o	+	Contact with horses, cats and dogs; previous i.c. tests positive to horse, guinea pig, cat and rabbit dander.
5	o	+	o	+	+	o	+	Contact with cow dander, previous i.c. tests positive to horse, guinea pig, cat and rabbit dander.
6	o	o	o	o	o	o	dubious	
7	o	o	o	o	+	o	+	Contacts: cat and dogs.
8	o	o	o	+	o	o	+	Contact: guinea pig (pet animal).
9	o	o	o	o	o	o	+	
10	o	o	o	o	o	o	+	
11	o	+	o	o	o	o	+	Contacts: cats and dogs.

Though, in the first group of patients, the correlation between the history of contacts and skin-reactivity to the respective dander allergens was poor, the results of Table 4 show that the skin reaction can occasionally be highly specific indeed. This is especially evident in patients 2 and 8, where the test reactions to the coded allergens, in retrospect, correlated well with the clinical history. The negative reaction of patient 5 to the cow dander extract may be attributed to a very low allergen content of the nondialysable material (see below). The test results in some subjects (no. 3, 4 and 5) who had also been included in the first series (a), did not correspond entirely with the intradermal tests performed previously.

TABLE 5

Results of intradermal tests with different (coded) dander allergens (20 µg/ml) and purified house dust allergen (2 µg/ml) in a third series of patients. Key: + weal no more than 5 mm diameter with erythema; 2+ weal 5-8 mm and erythema; 3+ weal 8-12 mm, with erythema and pseudopods. P = cow dander allergen; Q = dog; R = cat; S = guinea pig; T = horse.

Patient	P	Q	R	S	T	House dust
1	—	2+	3+	3+	2+	2+
2	—	—	—	—	—	—
3	—	—	—	—	—	—
4	—	—	—	—	—	—
5	—	+	+	—	+	+
6	—	+	3+	+	2+	2+
7	—	+	2+	—	+	2+
8	—	—	—	—	—	—
9	—	+	3+	—	+	2+
10	—	+	3+	+	+	2+
11	—	—	—	—	+	2+
12	—	—	+	—	+	2+

c) *Third series*

The above two groups of patients were investigated at the Paris Pasteur Institute. The subjects examined in the third series were patients attending the Out-Patient Department of

Allergy at Utrecht. The dander allergens were again submitted under code number and were evaluated by intradermal assay at 20 $\mu\text{g/ml}$. The results collected in Table 5, demonstrate a tendency in most patients to react to each of the dander allergens, though in later investigations some striking cases of isolated allergies have been observed.

From the overall results of these pilot studies we gained the impression that two types of responsiveness occurred in our patients. The predominant situation was that positive skin reactions occurred to several different dander allergens, which did not correlate well with a history of animal contacts. Occasionally, however, isolated skin reactions were observed—not necessarily in typically atopic patients—to one special dander extract; in such cases, this 'specific' hypersensitivity usually fitted well with a history of sensitization to the particular animal species.

In vitro tests

As shown in two recent reports (8, 9), atopic allergens are capable of inactivating human, but not guinea pig, complement in fluid phase. The inactivating capacity of individual allergens is related to the presence within the molecular framework, of 'allergenic' sites of lysine-sugar linkages. These structural sites have also been implicated as being responsible for eliciting non-allergen-specific skin reactions in man, by priming a universal mediator system of varying susceptibility among different people (5, 6, 8, 9).

The dander allergens employed in the present studies were examined for their capacity to induce *in vitro* consumption of human serum complement, by way of the simple technique described in the methods section. Fig. 2 collectively shows the experimental results obtained with a single serum sample (of patient 6, Table 5, who had not been desensitized to any of these dander allergens); similar results were obtained with other serum samples. It follows from this figure that dander allergens, like other atopic allergens (8), can induce consump-

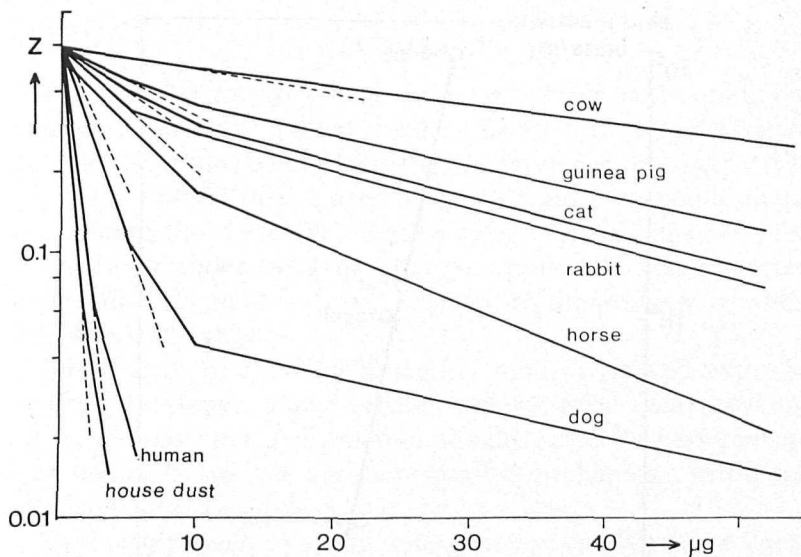


Fig. 2.

Graph showing the loss of human complement from the serum of patient 6, Table 5, by incubation under standard conditions (methods section) with house dust and dander allergens. Complement loss has been expressed in decreasing $Z = -\ln(1-y)$ values. (13, 16).

tion of human complement. Moreover, the different activities of the various dander allergens in this complement test system roughly coincide with their potencies in skin tests.

The C-inactivating power of individual allergenic extracts was compared by plotting complement-titers in the presence (Z_a) and absence (Z_o) of allergen as Z_a/Z_o ratios on a linear scale against allergen concentration on a logarithmic scale; by extrapolation to 100 per cent loss of haemolytic complement, this permitted comparing values for the *in vitro* activity of the different allergens.

The graph of Fig. 3 clearly demonstrates that there is a good correlation between the C-inactivating power of various dander allergens and the estimated mean skin-reactivities in units/mg calculated over the whole population of the first series of patients (Tables 2 and 3). It is of note that the "dose-

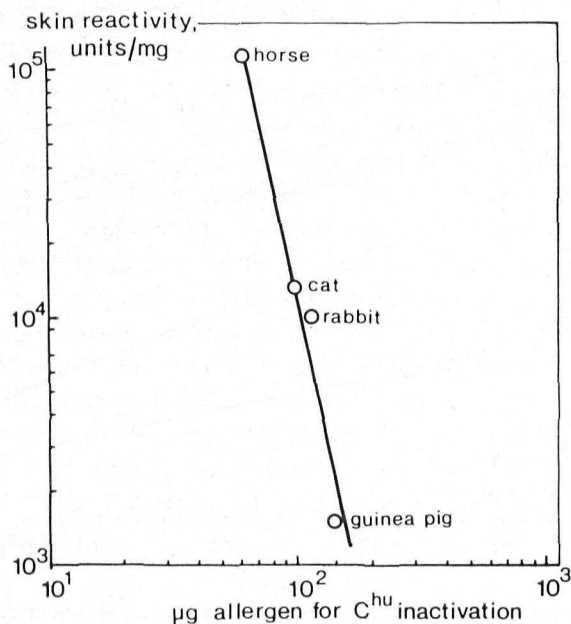


Fig. 3.

Relationship between *in vitro* capacity to inactivate complement in the serum of a single patient (expressed as μg of allergen required for 100 per cent complement inactivation under standard conditions), and the mean skin-reactivity (in units/mg) of the same allergens in a group of patients (series 1, Tables 2 and 3).

response" relationship depicted in Fig. 3 is very steep. This has also been found with other allergens; the effect may be due to amplifying sequential reactions in the skin, which do not proceed in the *in vitro* assay.

Taken together with the observation that the human dander allergen invariably produced positive skin reactions in our patients, and noting that the poor *in vitro* activity of our cow dander sample appears to correlate with its relative impotency in skin tests, the results of complement-inactivation measurements strengthen our conviction that the *in vitro* tests are a reflection of the non-allergen-specific action of the dander allergens.

DISCUSSION

The extremely low yields of total extractable and non-dialysable material from animal dander material has not permitted us to attempt purifying the allergens. Even though wide variations were noted in the percentage of total extractable material among the different danders (Fig. 1), this does not tell us anything about the true *allergen content* of the extracted material. This point is directly related to the problem of allergen standardization.

The results of the clinical studies tend to fit well with the concept developed along various experimental routes by one of us, namely that a distinction should be made between immunologically specific and non-specific mechanisms in human atopic disease (4, 5, 6, 8, 9).

In this approach to atopy, allergens are considered to exert a dual action. On the one hand, they may prime a universal mediator system, involving human serum complement *in vitro*, by virtue of a common 'allergenic determinant', or 'active site' of lysine-sugar bonding, which is not specific for the carrier macromolecule. Second, the specific *antigenic* determinants of an allergen molecule may induce the production of allergen-specific antibodies, probably of the IgE class. The clinical evidence presented in this paper indeed demonstrates that, whereas many patients react to several unrelated dander allergens, apparently in a non-specific fashion, isolated cases may occur of immunologically highly significant *specific* sensitization. The latter observation rules out inter-species cross-reactivities among dander allergens as an explanation for the multiple sensitivities observed in the other patients.

It is extremely difficult, in patients who display multiple skin reactivity to various distinct dander allergens, to decide on the mere basis of skin test results whether a positive response must be interpreted in terms of true immunological sensitization to the allergen, or whether this response just reflects the sensitivity of an underlying mediator system to the 'active sites' carried by the allergen. We feel that the future

clinical interpretation of such skin reactions should be accompanied by the results of appropriate *in vitro* tests.

If the allergens exert a dual action, one by virtue of common biologically active sites detecting the patient's intrinsic sensitivity of a universal mediator system, and the other based on the interaction with allergen-specific (IgE) antibody, it becomes obvious that two *in vitro* methods and two methods of standardization will be required. The results shown in the graph of Fig. 3, as well as results obtained with other allergens (8, 9) demonstrate, that the *in vitro* complement consumption test is a reliable method for evaluating and standardizing the non-allergen-specific biological activity of allergens; it may also aid in establishing the allergen content of crude allergenic extracts. Conversely, a single standard allergen will be suitable to determine the sensitivity *in vitro* of the complement system in the serum of individual patients. It should be remarked here that current studies with the sera from house dust and grasspollen sensitive patients indicate no correlation between serum complement sensitivity and total or specific IgE titers established by RAST.

As regards the *specific immunological sensitization* to special dander 'antigens' in patients, recourse must be had to methods for appraising the level of specific antibody. The radio-allergo-sorbent test discussed by Ceska and colleagues (10, 11, 18) may in such cases be a valuable tool. These different techniques may in the future offer two laboratory parameters, one for the 'atopic status' and one for the 'specific sensitivities', which might aid in establishing an accurate clinical diagnosis of animal dander sensitivity.

SUMMARY

Several different animal dander materials have been extracted, and the yields and analyses of total nondialysable substance have been compared. Various coded dander extracts were examined by prick and intradermal tests in atopic individuals; the allergens were also screened for their capacity to induce

loss of haemolytic complement from human allergic serum. Wide variations were noted in the percentage of extractable material among the different danders. Many patients reacted to skin tests with several unrelated dander allergens, and these skin responses did not correlate with a history of animal contacts. However, isolated cases were observed of immunologically highly specific sensitizations. Calculated mean skin reactivities of the allergens correlated well with the capacity to inactivate human complement in fluid phase *in vitro*. It is suggested that the allergens may exert a dual action, both unspecific and/or immunologically specific.

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