

Affinity purification of a major and a minor allergen from dog extract: Serologic activity of affinity-purified *Can f I* and of *Can f I*-depleted extract

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Most dog-allergic patients react to a major 25 kd component on sodium dodecyl sulfate blots, Can f I (Ag 13). We initially raised monoclonal antibodies (Cf-3 and Cf-2) reactive with IgE-binding components distinct from Can f I. After a slight modification, we immunized other strains of mice and produced monoclonal antibodies coded Cf-1a and Cf-1b reactive with Can f I. We affinity purified the allergens, Can f I and "dog allergen 2" with Cf-1a and Cf-2 ascites, respectively, and house dust-rich dog dander. Comparison of purified Can f I with dog saliva in RAST demonstrated that Can f I is a potent allergen for most dog-allergic patients (average response, 70%). After depletion of dog saliva of Can f I, a slightly lower contribution for Can f I was found, but the overall results supported the conclusion that Can f I is a major allergen in dog saliva. Comparison of purified dog allergen 2 with dog dander in RAST demonstrated that dog allergen 2 is less important for dog-allergic patients (average response, 23%). We radiolabeled the purified allergens and developed assays to measure Can f I and dog allergen 2 in allergen extracts and dust samples. Dog saliva was a strong allergen source, dog urine and feces contained very little of the allergens, and both allergens were found to a variable degree in the nine dog breeds tested. (J ALLERGY CLIN IMMUNOL 1991;87:1056-65.)

In addition to house dust mites, dogs are an important source of indoor allergens. At least 31% of the houses randomly selected contained dogs as pets.¹ In our outpatient population, 16% of the houses contained dogs compared to 27% of houses with cats. Even in schools² and houses without pets,³ dog allergen was detected. Dog-dander allergy is reported to occur in about 15% of a young adult population.⁴ In our own patient population, the incidence of dog-dander allergy is 17% compared to an incidence of cat-dander allergy of 24%. This study was done in 1988 and 1989 in a group of 592 patients with rhinoconjunctivitis and/or asthma. Therefore, it was important to investigate the allergen(s) in dog extracts

to which most dog-allergic patients react and to investigate whether there is only one important major allergen, as found in cat allergy,⁵⁻⁷ or several major allergens, as was found by depletion studies for the house dust mite by van der Zee et al.⁸

Varga and Ceska⁹ already characterized commercial dog extracts by isoelectric focusing and RAST and detected major common component(s) between isoelectric point 4.3 to 4.7. However, additional components in other isoelectric point ranges were found in another dog extract, probably from additional dog breeds. Løwenstein¹⁰ and Ford et al.¹¹ confirmed the existence of an important allergen (Ag 13 and Ag 8, respectively) using CRIE techniques.

In this study, we developed MABs directed to different dog antigens using dog dander and dog saliva as important allergen sources, as previously reported.¹²⁻¹⁵ After the production of the MABs, we affinity purified two proteins analogous to the procedure we used for the purification of *Fel d I* with house dust as allergen source.^{6,7} By means of RAST, we investigated the serologic importance of these purified proteins in a panel of dog-allergic patients. Furthermore, we radiolabeled the two dog allergens and developed a competitive radioimmunoassay to measure the dog allergens in dust samples and in allergen extracts.

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Abbreviations used

<i>Can f</i> I:	<i>Canis familiaris</i> 1 (Ag 13), a major allergen from dog extracts
MAb:	Monoclonal antibody
Cf-1a, Cf-1b:	Code of MAbs directed to <i>Can f</i> I
Cf-2:	Code of MAbs directed to dog allergen 2
Cf-3:	Code of MAbs directed to a high molecular weight protein from dog dander
Cf-s:	Code of MAbs directed to dog serum
CRIE:	Crossed radioimmuno-electrophoresis
Dog allergen 2:	Minor allergen from dog extracts with a molecular weight of 27 kd
<i>Fel d</i> I:	<i>Felis domesticus</i> allergen 1. <i>Fel d</i> I is the name proposed by the International Union of Immunological Societies for Cat 1 or Ag 4
HAL:	Haarlems Allergen Laboratorium, Haarlem, The Netherlands
PBS:	Phosphate-buffered saline
PBS-AT:	PBS containing 0.3% wt/vol of bovine serum albumin and 0.1% (vol/vol) of Tween 20 and 5 mmol/L of NaN ₃
SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
Ab:	Antibody
Ag:	Antigen

MATERIAL AND METHODS

Allergen sources

Dog-dander extract was obtained from HAL. Dog saliva (after stimulation with atropine), serum, and urine (voided and filtrated) were collected from two beagles. Saliva was freeze-dried and reconstituted in PBS. Dog feces were collected "freshly" and extracted overnight at 20% (wt/vol) with a phenol buffer containing 0.037 mol/L of NaH₂PO₄, 0.036 mol/L of Na₂HPO₄, 0.13 mol/L of NaCl, and 0.5% (wt/vol) of phenol at pH 6.8.

Hairs from nine different dog breeds were collected by brushing the dogs, and the proteins were extracted overnight at 2.5% (wt/vol) with phenol buffer. House dust was collected with a standard vacuum cleaner, and 10% (wt/vol) extracts were made in phenol buffer. After filtration and adding of 0.5 mg/ml of NaN₃, the extracts were stored at 4° C until use.

Abs

Polyclonal Abs directed to dog dander were kindly provided by Dr. C. Schou (Allergologisk Laboratorium, Copenhagen, Denmark). Polyclonal goat-antimouse immunoglobulin and sheep anti-IgE were obtained from the De-

partment for the Production of Immune Reagents (Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands). These Abs were affinity purified and labeled with ¹²⁵I by the chloramine-T technique. The labeling was performed according to the method of Hunter and Greenwood.¹⁶

Other reagents

Radioimmunoassay buffer (PBS-AT) contained PBS (0.01 mol/L of phosphate and 0.14 mol/L of NaCl) with 0.3 mg/ml of bovine serum albumin (Poviet, Amsterdam, The Netherlands), 10 mmol/L of ethylenediaminetetraacetic acid, 5 mmol/L of NaN₃, and 0.1% vol/vol of Tween 20 (Baker, Deventer, The Netherlands) at pH 7.4. CNBr-activated Sepharose 4B and protein A-Sepharose were obtained from Pharmacia Fine Chemicals (Uppsala, Sweden).

Patients

Blood samples for RAST analyses were, in part, selected from samples submitted for a routine allergic evaluation in our laboratory and, in part, from the Department of Pulmonary Diseases of the Academic Medical Center (Amsterdam). Forty-one sera were selected for the RAST tests; these sera were characterized by a RAST score to dog dander (HAL) of >5% bound anti-IgE.

Immunization procedure

Three immunization protocols were used to produce MAbs to three different dog allergens: (1) immunization of Balb/c mice with 50 µg of freeze-dried and dialyzed dog-dander extract (HAL), (2) immunization of Balb/c mice with 50 µg of freeze-dried and dialyzed dog saliva (corresponding to 175 µl of saliva), and (3) immunization of four mouse strains, among other Balb/b, with 500 µg of freeze-dried dog saliva from which a 27 kd protein was removed with the MAbs from the second fusion.

For the depletion, 35 ml of saliva was incubated batch-wise with 4 gm of CNBr-activated Sepharose containing 4 ml of MAb ascites. In all three immunization protocols, the mice were injected subcutaneously with 50 µl of the dog extract in complete Freund's adjuvant and boosted after 6 weeks with the same dose in incomplete Freund's adjuvant. An intravenous booster with the same amount of the allergen was administered 4 days before removal of the spleen.

Production of MAbs

Hybridization was performed according to the method of Kohler and Milstein^{17, 18} with some modifications according to the method of Astaldi et al.¹⁹ Ab-producing hybrids were selected by means of a RAST with Sepharose-coupled dog-dander extract and ¹²⁵I-labeled goat-antimouse IgG. Positive wells were additionally tested with an indirect RAST system. Sepharose-coupled MAbs were incubated with dog-allergen extract, allergen binding was quantitated with human IgE Abs, and ¹²⁵I-labeled sheep anti-IgE. Cloning was performed by repeated limiting dilution.

MAbs, coded Cf-1a (5B3) and Cf-1b (6E9) were obtained from Balb/b mice immunized with depleted saliva. To ob-

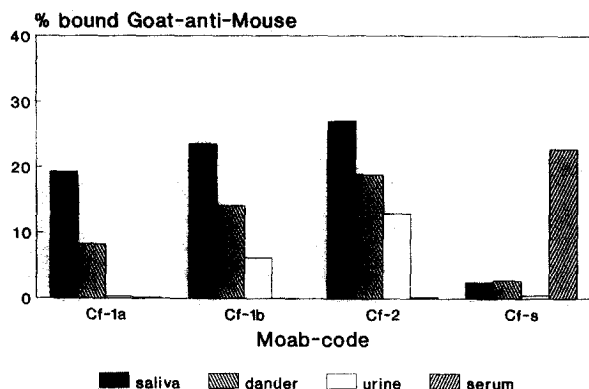


FIG. 1. Specificity of the MAbs directed to dog allergens. Data are expressed as percentage-bound radioactivity (goat-anti-mouse). The MAbs were directed to *Can f* 1 (code Cf-1a and Cf-1b), dog allergen 2 (code Cf-2), and dog serum (code Cf-s), respectively.

tain ascites, Balb/c mice were irradiated (300 rad) and pristane treated before cell transfer. MAb Cf-2 (7E7) was obtained from a Balb/c mouse immunized with dog saliva. MAbs coded Cf-2 (7C7) and Cf-s (5A3) were obtained from a Balb/c mouse immunized with dog dander.

Specificity of MAbs

1. Two hundred fifty microliters of allergen Sepharose (in a concentration of 2 mg/ml) was incubated overnight with 50 μ l of 1:3000 diluted ascites. After a washing procedure, radioactive goat-antimouse IgG was added, and another overnight incubation followed. After another washing procedure, percentage-bound radioactivity was measured.
2. SDS-PAGE Western blotting: polyacrylamide electrophoresis (12.5 $\times$ 14.5 cm, 12.5% gel) of 1 mg of freeze-dried dog saliva was performed under reducing conditions and blotted onto nitrocellulose, according to the method of Calkhoven et al.²⁰ Blots were blocked with PBS-AT. Each blot strip was incubated overnight with 200 μ l of patient's serum or ascites (1:1000 diluted) in 5 ml of PBS-AT. After an extensive washing, the blot strips were incubated overnight with 60,000 cpm of ¹²⁵I-labeled sheep-antihuman IgE and ¹²⁵I-labeled goat-antimouse IgG, respectively, in 5 ml of PBS-AT. After another washing and drying procedure of the strips, autoradiography was performed at -70° C for approximately 60 hours with Kodak X-OMAT S films (Eastman Kodak, France) with an intensifying screen.

Affinity purification of two dog allergens

With MAb Cf-1a. As a source of allergen, house dust containing dog dander was used. The allergen in the extract was adsorbed onto a phenyl-Sepharose column (600 ml, 1 mol/L of (NH₄)₂SO₄ solution); 72 ml of the fraction eluted with water (containing 1526 μ g of *Can f* 1) was adsorbed for 4 hours to 2.7 gm of Cf-1a Sepharose. For this purpose, ascites was precipitated with 50% saturated ammonium sulfate and dialyzed; 109 optical density units at 280 nm were

added to 2.6 gm of CNBr-activated Sepharose. Coupling efficiency was 91%. *Can f* 1 was eluted from the affinity matrix with 0.1 mol/L of glycine HCl, pH 2.5, and the fractions with the highest protein concentration, measured with extinction, were pooled. Neutralization was performed with 100 μ l of 1 mol/L of Tris buffer, pH 8.5. Protein content was determined according to the method of Pierce and Suelter²¹ with bovine serum albumin as reference.

With MAb Cf-2. The same procedures were used for the affinity purification of dog allergen 2. In this procedure, 36 ml of the phenyl-Sepharose eluate (water fraction containing 136 μ g of dog allergen 2) was adsorbed to 1.3 gm of Cf-2 Sepharose (again, 30 mg of ascites coupled per gram of Sepharose). Coupling efficiency was 93%. Elution with glycine HCl yielded approximately 73 μ g of dog allergen 2.

Labeled Ags

Allergens eluted from the affinity matrix were labeled with the chloramine-T technique.¹⁶ One millicurie was added to 10 μ g of *Can f* 1 and 4.3 μ g of dog 2 allergen, respectively. The labeling was followed by gel filtration over a column of ACA-54 (LKB, Bromma, Sweden). Immune reactivity was >50% with CNBr-activated Sepharose to which Ab-containing ascites was coupled.

RAST

RAST on a panel of 41 dog-allergic patients was performed with 250 μ l of allergen Sepharose (in a concentration of 2 mg/ml) and 50 μ l of patient's serum in the presence of 10% (vol/vol) of dog serum. After an overnight incubation and washing with saline, ¹²⁵I-labeled sheep anti-IgE was added and then another overnight incubation. The results are expressed as percentage-bound labeled anti-IgE after correction for nonspecific binding.²² The results were converted into RAST units where this was indicated.

We coupled equal amounts of *Can f* 1 (13.2 μ g/100 mg of activated Sepharose), either affinity-purified *Can f* 1 or as whole dog saliva. Similarly, we coupled equal amounts of dog allergen 2 (14 μ g/100 mg of activated Sepharose), either affinity-purified dog allergen 2 or as whole dog-dander extract. Coupling efficiency was monitored by incubating allergen Sepharose with 50 μ l of Cf-1 and Cf-2 MAbs, respectively. After a washing and a second incubation with labeled goat-antimouse immunoglobulin, percentage-bound radioactivity was measured.

The sera were also tested on dog-serum Sepharose (200 μ l coupled per 200 mg of activated Sepharose).

Depletion of *Can f* 1

For the depletion of 1.5 ml (concentration, 1 mg/ml) of freeze-dried dog saliva (containing 50 μ g of *Can f* 1), we coupled 10 mg of ammonium-sulfate-precipitated Cf-1b (6E9) ascites to 500 mg of CNBr-activated Sepharose. The final volume was 3 ml.

As a control, we incubated dog saliva with glycine-inactivated Sepharose. After batchwise overnight incubation, supernatants were collected, called "*Can f* 1-depleted" and "sham-depleted" supernatant, respectively. Next, we coupled 660 μ l of the supernatants to 100 mg of activated Sepharose. The supernatants were equivalent regarding the

TABLE I. Flow sheet of the affinity purification of two dog allergens

	<i>Can f</i> I	Dog allergen 2
MAB used	Cf-1a (5B3)	Cf-2 (7E7)
Allergen adsorbed	1393 µg	127 µg
Allergen eluted	311 µg	73 µg
Yield	22%	50%
E ₂₈₀ peak fraction	0.120	0.075
Protein content	40 µg/ml	14 µg/ml

E₂₈₀, Optical density at 280 nm.

dog allergen 2 concentration (36.2 and 39.4 µg/ml, respectively). The two allergosorbents were used to test the effect of depletion in 37 sera, with normal RAST procedures in the presence of 10% (vol/vol) of dog serum. As control, the sera were tested on purified *Can f* I Sepharose, adjusted to contain equal amounts of *Can f* I as was still present in the *Can f* I-depleted allergosorbent. The results were corrected for this residual *Can f* I reactivity.

Allergen determinations

***Can f* I.** Fifty microliters of an allergen-containing sample was mixed with 50 µl of a 1:2000 dilution of rabbit anti-serum to dog dander (Allergologisk Laboratorium). Incubation was performed for 2 hours at room temperature. Then, 500 µl of protein A-Sepharose (concentration, 1 mg/ml) and 50 µl of ¹²⁵I-labeled *Can f* I (0.5 ng per test) were added, and the suspension was incubated overnight. After centrifugation and a washing of the Sepharose, bound radioactivity was measured. As a reference material, we used affinity-purified *Can f* I that contained 40 µg/ml, according to the protein-determination method of Pierce and Suelter.²¹ The standard curve of the *Can f* I assay was repeated five times; the coefficient of variation was 5% to 10%, depending on the antigen level.

Dog allergen 2. The same procedures accounted for the dog allergen 2 assay with a few modifications. A 1:5000 dilution of rabbit antiserum to dog dander was used, and 50 µl of ¹²⁵I-labeled dog allergen 2 (0.25 ng per test) was added for detection.

RAST-inhibition assay. For the measurement of the potency of the dog-hair extracts, we initially used a RAST-inhibition assay. In this assay, 110 µl of a serum from a dog-allergic patient was incubated for 2 hours with an equal volume of a dilution of dog-hair extract. From this mixture, 100 µl was incubated overnight with 100 µl of a trinitrophenylated dog allergen and anti-trinitrophenyl beads, with the modified RAST procedure, as previously described.²³ The concentration of dog-hair extract eliciting a 50% inhibition was calculated. Data were expressed as potency relative to dog-dander extract (HAL).

RESULTS

Specificity of MABs

Direct mouse RAST. The reactivity of the MABs resulting from immunization with dog dander, dog

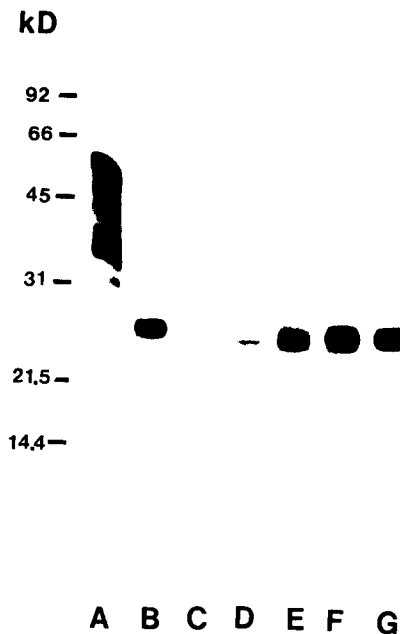


FIG. 2. Immunoblot of dog saliva incubated with MABs and sera from dog-allergic patients. Lane A, MAB Cf-3; lane B, MAB Cf-2; lane C, MAB Cf-1a; lane D, MAB Cf-1b; and lanes E-G, three dog-allergic patients.

saliva, and depleted dog saliva, respectively, toward different dog allergens is illustrated in Fig. 1. No reactivity toward cat allergens was observed in this test system.

SDS-PAGE. The results of the immunodetection of the patients' sera and the MABs on the dog-saliva blot strips are illustrated in Fig. 2. It was found that MAB Cf-1 (clone 5B3 as well as clone 6E9) reacted with a 25 kD protein (*Can f* I) that also elicited a reaction in most dog-allergic patients. MAB Cf-2 reacted with a 27 kD protein (dog allergen 2). MAB Cf-3 reacted with higher molecular weight structures compared to IgE Abs of dog-allergic patients and will not be discussed further. By means of an radioimmuno-inhibition assay (data not presented), it was found that the binding of clone 5B3 (Cf-1a) was blocked >90% with clone 6E9 (Cf-1b).

Affinity purification of two dog allergens

The results of the affinity purification of *Can f* I and dog allergen 2 are presented in Table I. Protein yields were 40 µg and 14 µg/ml of *Can f* I and dog allergen 2, respectively. The purity of the affinity-purified material was assessed by iodination, SDS-PAGE, and autoradiography. This assessment demonstrated only a single band.

Can f I RAST

To establish the contribution of isolated allergens to the dog RAST, we coupled the same amounts of

TABLE II. IgE response of 41 dog-allergic patients

Patient No.	A	B	C	D	E	F
1	1	23	20	8	37	13
2	12	19	20	1	33	1
3	1	16	1	14	19	1
4	1	20	11	15	20	10
5	1	31	20	12	42	19
6	1	15	15	0	21	3
7	2	6	4	1	17	2
8	2	4	4	0	20	1
9	41	1	1	0	12	3
10	1	26	28	0	32	1
11	1	34	12	28	45	21
12	0	20	10	12	24	3
13	1	10	13	1	20	7
14	1	23	18	14	32	1
15	1	22	18	9	27	9
16	2	22	26	3	32	6
17	1	38	34	21	44	1
18	53	1	0	0	47	39
19	49	1	1	1	20	1
20	2	47	46	18	55	18
21	1	15	1	14	17	1
22	12	5	2	4	21	3
23	0	41	28	35	51	48
24	0	5	2	6	12	3
25	2	35	21	34	41	27
26	1	20	20	2	30	14
27	6	22	17	13	29	4
28	17	29	15	22	48	18
29	1	26	23	2	34	1
30	2	21	21	2	30	6
31	2	36	34	3	48	1
32	0	20	21	0	27	1
33	1	4	5	0	6	1
34	2	37	23	29	43	33
35	52	1	1	1	35	1
36	0	14	13	2	19	5
37	0	36	18	29	36	1
38	38	16	16	5	32	4
39	1	8	9	3	16	1
40	0	22	10	17	44	13
41	1	16	7	15	41	11

A, Dog serum; B, glycine-absorbed dog saliva; C, affinity-purified *Can f* I; D, *Can f* I-depleted dog saliva (corrected, see text); E, dog dander (equivalent dog allergen 2 concentration, as in F); F, affinity-purified dog allergen 2.

The results are expressed as raw data: percent of input anti-IgE, meaning that these data in columns C and D cannot be expected to elicit the results in column B.

purified allergens as was found in the raw material (saliva and dander). As monitored by mouse RAST with MAbs Cf-1 and Cf-2, it was confirmed that the amounts of purified *Can f* I and dog allergen 2, which were coupled to Sepharose, were equal to the amounts coupled with dog saliva and dog dander, respectively.

The IgE response of the 41 dog-allergic patients directed to these allergosorbents is presented in Table

II. The RAST with whole dog saliva compared to the RAST with purified *Can f* I of 37 dog-allergic patients is illustrated in Fig. 3, A. The average ratio (anti-IgE bound to purified *Can f* I versus anti-IgE bound to whole dog saliva) was 0.70 (range, 0.05 to 1.2). Twenty-two patients of 37 reacted with >50% of the IgE response with purified *Can f* I, whereas 18 patients (50%) reacted with >75% of the IgE response. From

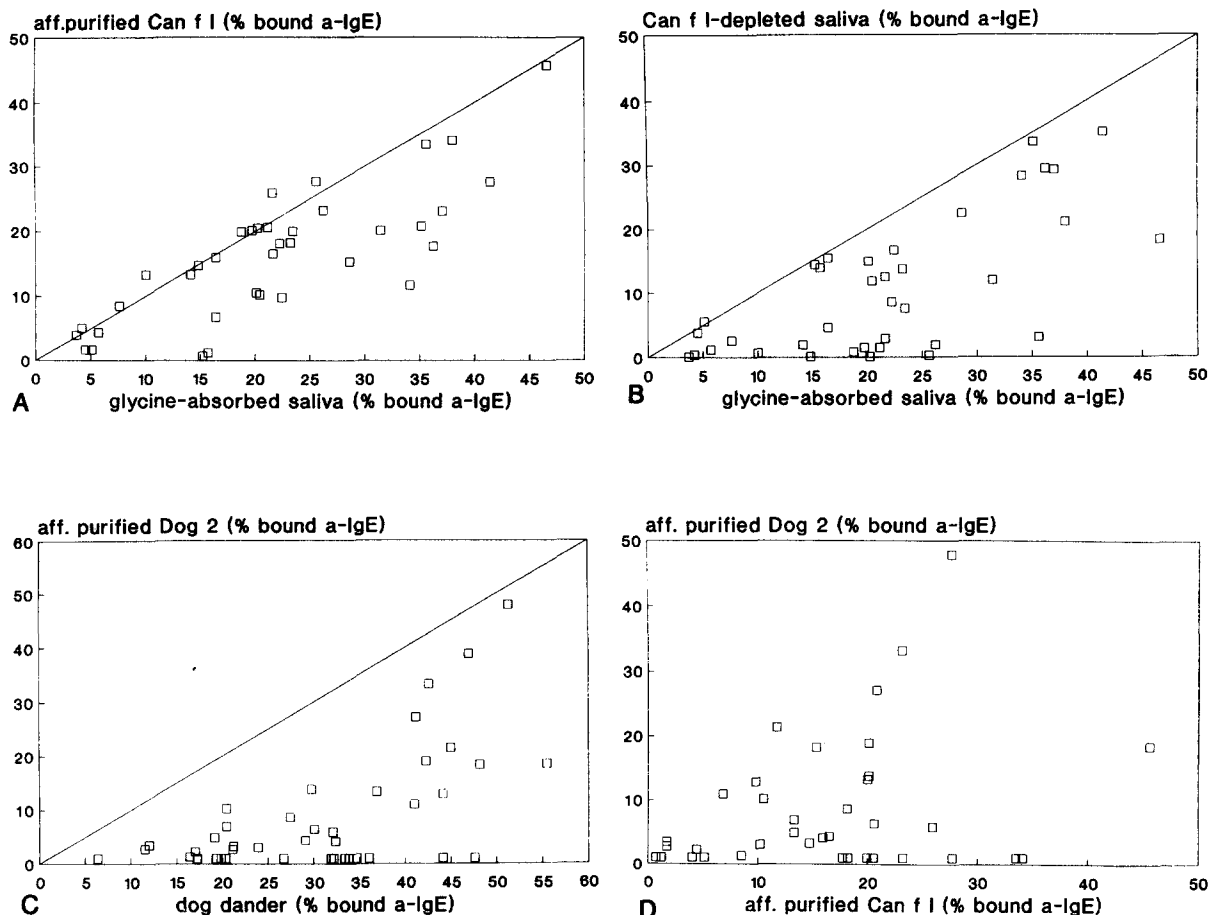


FIG. 3. **A**, RAST with glycine-absorbed dog saliva (*X* axis) compared with the RAST with purified *Can f I* (*Y* axis) on a panel of 37 dog-allergic patients, tested in the presence of 10% (vol/vol) dog serum. *Can f I* concentrations on both allergosorbents were equal, as monitored with MAb Cf-1a and Cf-1b. Data are expressed as percentage-bound ¹²⁵I-labeled anti-IgE. **B**, RAST with glycine-absorbed dog saliva (*X* axis) compared with the RAST with *Can f I*-depleted dog saliva (*Y* axis) on a panel of 37 dog-allergic patients, tested in the presence of 10% (vol/vol) dog serum. Data are expressed as percentage-bound ¹²⁵I-labeled anti-IgE. **C**, RAST with dog-dander extract (*X* axis) compared with the RAST with purified dog allergen 2 (*Y* axis) on a panel of 41 dog-allergic patients. Dog allergen 2 concentrations on both allergosorbents were equal, as monitored by MAb Cf-2. Data are expressed as percentage-bound ¹²⁵I-labeled anti-IgE. **D**, RAST with purified *Can f I* extract (*X* axis) compared with the RAST with purified dog allergen 2 extract (*Y* axis) on a panel of 37 dog-allergic patients. Data are expressed as percentage-bound ¹²⁵I-labeled anti-IgE.

these data, we conclude that we have isolated a major allergen from dog extract.

Depletion of *Can f I*

The concentration of *Can f I* allergen in the sham-depleted and the *Can f I*-depleted supernatants was 32.0 and 1.7 µg/ml, respectively, demonstrating that after incubation of dog saliva with the ascites, 94.7% of the *Can f I* allergen was depleted. The RAST with the *Can f I*-depleted dog saliva compared to the sham-depleted dog saliva in the presence of dog serum is illustrated in Fig. 3, *B*. For the calculation, the RAST units of the *Can f I*-depleted allergosorbent were cor-

rected for the RAST units of the diluted *Can f I* Sepharose to correct for the remaining *Can f I* still present after the depletion.

Depletion of *Can f I* allergen from dog saliva was demonstrated to result in an average reduction of 57% of the IgE response in 37 patients; 21 patients revealed a reduction of >50%, whereas 14 patients (38%) revealed a reduction of >75% after depletion of *Can f I*.

Dog allergen 2 RAST

The RAST with dog-dander extract compared to the RAST with purified dog allergen 2 of 41 patients

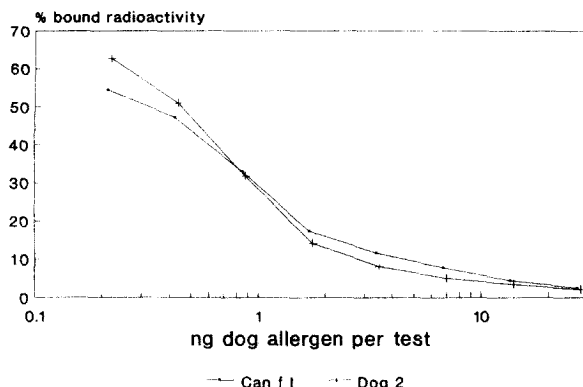


FIG. 4. Dilution curve for the reference preparation (dog-dander extract, HAL) regarding the *Can f I* and dog allergen 2 determination. On the *X axis*, the concentration of dog allergen in nanograms per test; on the *Y axis*, the percentage-bound radioactivity.

is illustrated in Fig. 3, C. The mean ratio (anti-IgE bound to purified dog allergen 2 versus anti-IgE bound to whole dog dander) was 0.23 (range, <0.05 to 0.93). In Fig. 3, D, the correlation between the IgE response directed toward *Can f I* and the IgE response directed toward dog allergen 2 is low.

Allergen determinations

The dose-response curve for *Can f I* and dog allergen 2 of a dog-dander extract (HAL) is illustrated in Fig. 4. Concentrations of dog allergens in different dog extracts and house dust samples are illustrated in Fig. 5. All houses with dogs contained considerable amounts of the two dog allergens; even in the mattresses, dog allergens were found. The dog-allergen concentrations in the 27 hair extracts of nine dog breeds varied widely.

From Fig. 6, it was concluded that there is a good correlation between the *Can f I* allergen determination with the labeled Ag-binding assay and the RAST-inhibition test with a patient (No. 1) with a high IgE response to *Can f I*. The correlation between the dog allergen 2 determination, with the labeled Ag-binding assay and the RAST-inhibition test with the same patient, was low, reflecting the disadvantage of use of a serum with its own allergen specificity in allergen determinations.

DISCUSSION

MAbs to dog allergens

In our attempt to produce MAbs to dog allergens, we experienced problems. The first fusions with Balb/c mice resulted in MAbs that recognized a 27

kd protein capable of binding IgE Abs of dog-allergic patients, but clearly distinct from the 25 kd protein that was strongly stained by IgE of most patients on SDS-PAGE immunoblots. From two subsequent fusions, we concluded that this 27 kd protein was very immunogenic for Balb/c mice; therefore, we decided to deplete dog-saliva extract for this protein. Next, we found that this extract was of very low antigenic potency for mice; none of the eight Balb/c mice immunized with this extract reacted, and only one of four A/J mice, one of four Black 10 mice, and two of four Balb/b mice demonstrated a positive response after repeated immunization.

With a Balb/b mouse for the fusion, we were able to obtain MAbs (coded Cf-1a and Cf-1b) reactive with the 25 kd component to which most dog-allergic patients reacted. The MAbs were highly reactive in dot-blot experiments to *Can f I* or Ag 13²⁴ affinity purified with polyclonal monospecific Abs. These experiments were kindly performed by Dr. C. Schou (Copenhagen). With these MAbs, we affinity purified *Can f I* and dog allergen 2 from house dust rich in dog dander and hair. We used house dust as allergen source because of our previous good results with the purification of *Fel d I*.⁶ We investigated the serologic relevance of these dog allergens with purified allergen and allergen-depleted extracts in RAST.

Contribution of *Can f I* to allergenic activity of dog-allergen extracts

We found in the RAST that the average IgE response of 37 dog-allergic patients toward purified *Can f I* was 70% relative to the IgE response toward whole dog saliva. Sixty percent of the patients reacted with >50% of their IgE, and even 49% of the patients reacted with >75% of their IgE with *Can f I* allergen. To rule out IgE Abs to dog-serum components, present in approximately 20% of the dog-allergic patients, we performed the RAST experiments in the presence of dog serum.

Next, dog saliva was treated with MAb Cf-1 coupled to Sepharose, and this resulted in 95% depletion of *Can f I*. RAST with this depleted extract resulted in an average reduction of 57% of the IgE response. In 38% of the patients, the reduction was >75%. In our analysis, we corrected for the IgE Abs reactive with remaining traces of *Can f I*.

Histamine release (performed according to the methods described previously^{6, 25-27}) demonstrated that purified *Can f I* had biologic activity in a dog-allergic patient (preliminary results). We conclude that *Can f I* is a major allergen capable of provoking an IgE

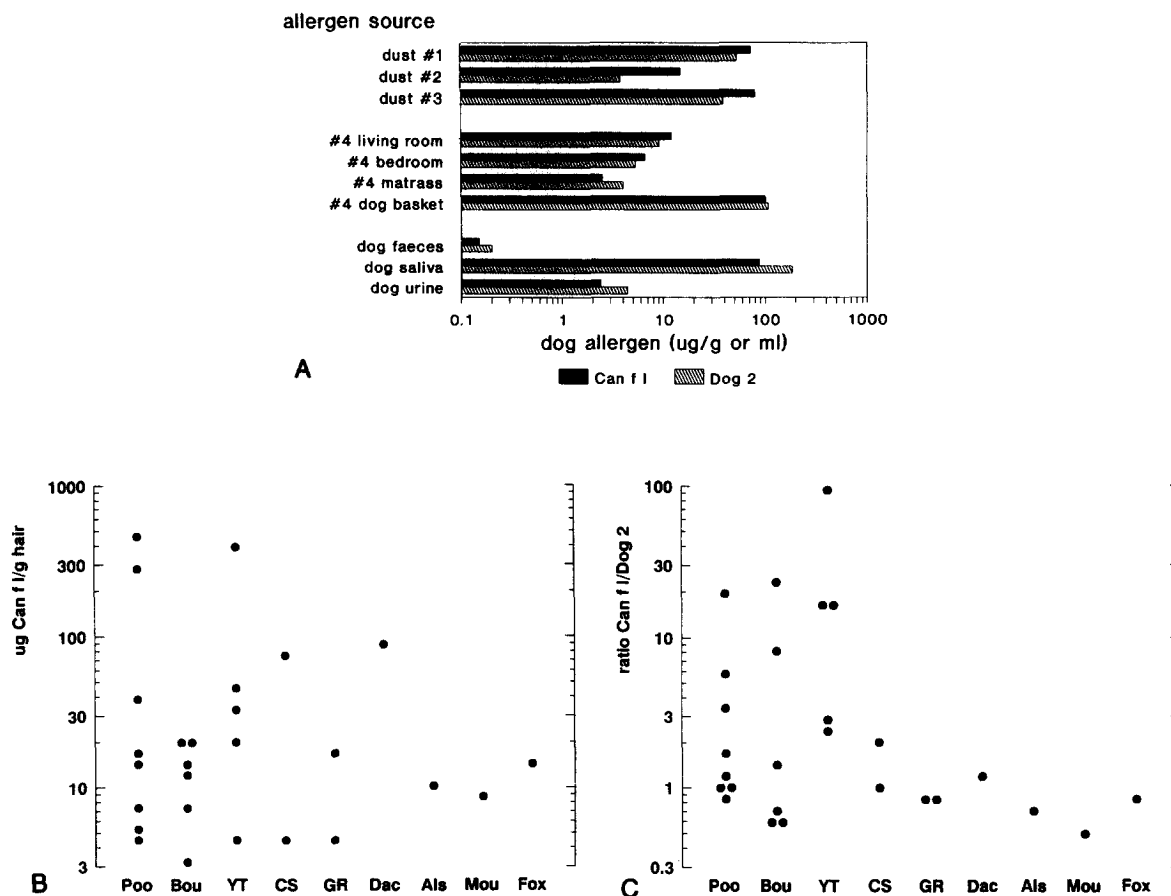


FIG. 5. A, Concentration of *Can f I* and dog allergen 2 in different extracts. Data are illustrated as micrograms of allergen per gram of dust or dog faeces and per milliliter of dog saliva and urine, respectively. Dust Nos. 1 to 4 were dust samples of houses in which dogs were kept. B, Dog-allergen concentrations in 27 hair extracts of nine dog breeds: Poodle (*Poo*), Bouvier (*Bou*), Yorkshire terrier (*YT*), cocker spaniel (*CS*), golden retriever (*GR*), dachshund (*Dac*), Alsatian (*Als*), mountain dog (*Mou*), and farmer's fox (*Fox*). B, Data are expressed as micrograms of allergen per gram of hair extracted for *Can f I*. C, Ratio of *Can f I*/dog allergen 2.

response in most dog-allergic patients. This component is identical to the protein described by Løwenstein¹⁰ and Schou and Løwenstein²⁴ as Ag 13 and by Ford et al.¹¹ as Ag 8. Løwenstein¹⁰ described the presence of 20 Ags in dog-dander extract, seven of which were of allergenic importance (CRIE with 16 dog-allergic patients), especially Ag 2 (albumin), Ag 6 (unidentified serum component), Ag 20 (immunoglobulin), and Ag 13 (a nonserum component) were relevant. However, none of these Ags appeared to be a major allergen (i.e., eliciting a strong reactivity with IgE in >50% of the patients). Ford et al.,¹¹ also using the technique of CRIE, identified 21 dander and serum allergens from dog-hair extract. Especially Ag 8, Ag 1, Ag 23 (IgG), and Ag 3 (albumin) appeared to be

of importance; however, no single major allergen could be qualified. Sixty-three percent of the sera bound at any intensity with Ag 8; only 32% of the sera bound strongly. Our finding that *Can f I* is a major allergen from dog is in contrast with their conclusions, and this fact can be due to differences in selection of human sera, the allergen sources used, and the techniques used.

Dog allergen 2

The average IgE response of 41 patients to purified dog allergen 2 was only 23% compared to the IgE response to whole dog-dander extract. There was no IgE response in 34% of the patients to dog allergen 2 and an IgE response of >50% in only 12% of the

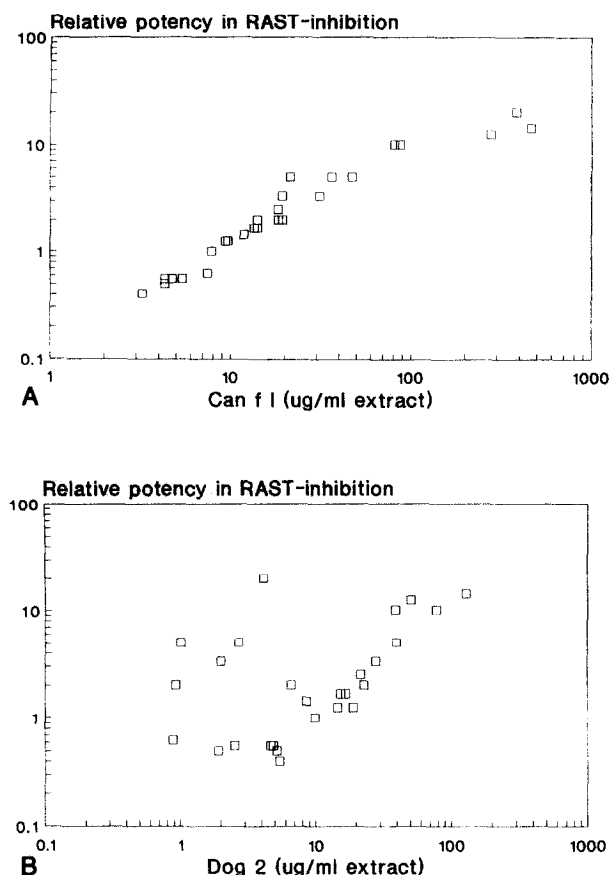


FIG. 6. Comparison of the dog-allergen determination with the labeled Ag-binding assay and the RAST-inhibition assay. **A,** *Can f I* (X axis). **B,** Dog allergen 2 concentration as established with the labeled Ag-binding assay, expressed in micrograms of allergen per milliliter of hair extracted. The concentration of the dog-breed extract needed to elicit a 50% inhibition of the dog RAST with a dog-allergic patient (Y axis), expressed as potency relative to freeze-dried dog-dander extract (HAL).

patients. Histamine release demonstrated that purified dog allergen 2 has biologic activity in a dog-allergic patient (preliminary data).

We conclude that we have affinity purified a second dog allergen with a high immunogenicity for Balb/c mice, but of minor importance for dog-allergic patients.

Dog-allergen exposure determination

An Ag-binding inhibition assay was developed for *Can f I* and dog allergen 2 determinations in dust samples and allergen extracts. Both allergens were found in dust samples of homes containing dogs and in 27 hair extracts of nine different dog breeds. Dog saliva appeared to be a strong allergen extract, containing 87 and 184 μg of *Can f I* and dog allergen 2 per milliliter, respectively, whereas dog urine and dog

feces contained very little allergen. The dog system resembles, in this way, the cat system, in which cat dander and, especially cat saliva, were the main sources of the major cat allergen, *Fel d I*.⁶

Although the MAbs directed to *Can f I* and dog allergen 2 were not reactive with cat allergosorbents, we found both dog allergens in all commercial cat-dander extracts tested. No dog allergens were detected in cat saliva or cat serum. In contrast, no *Fel d I* was found in dog-dander extracts, dog saliva, or dog serum. Only traces of *Fel d I* were detectable in 5/27 hair extracts from dogs living in a house together with cats.

Dog allergens in cat-dander extracts could be caused by a contamination or the presence of cross-reactive components in cats and dogs. The presence of cross-reactive components was first mentioned by Ohman et al.²⁸; these components were all serum allergens. Brandt and Yman,¹⁴ however, described the presence of a nonserum component with a molecular weight of 25 kd in dog-dander extract capable of inhibiting the cat-dander RAST. Contamination of cat-dander extracts with dog dander or the existence of dog allergens in cat acting as minor allergens for the cat could explain the extensive clinical cross-reactivity, as observed in the literature and in our outpatients; 88% of the patients positive in skin test and RAST to dog dander were also positive in both tests to cat dander. This interesting phenomenon is, at the present time, being further investigated.

We conclude that with the purified radiolabeled dog allergens we have a sensitive tool to examine whether there is a seasonal variation in dog-allergen exposure and the rate of disappearance of dog allergens after removal of the dog(s) from the home. Furthermore, it is of clinical importance to investigate the size of the particles containing dog allergens that become airborne and to establish some kind of arbitrary level of exposure above which atopic individuals become sensitized or start to have complaints of asthma, as has already been investigated for the house dust-mite and cat allergens.^{29, 30}

In this manner we can study more precisely the relationship between allergen exposure and dog hypersensitivity, as frequently observed in our outpatient population with asthma.

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REFERENCES

- Kjellman B, Petterson R. The problem of furred pets in childhood atopic disease. *Allergy* 1983;38:65-73.
- Dybendal T, Vik H, Elsayed S. Dust from carpeted and smooth floors. II. Antigenic and allergenic content of dust vacuumed from carpeted and smooth floors in schools under routine cleaning schedules. *Allergy* 1989;44:401-11.
- Wood RA, Eggleston PA, Lind P, et al. Antigenic analysis of household dust samples. *Am Rev Respir Dis* 1988;137:358-63.
- Haahtela T, Jaakonmaki I. Relationship of allergen-specific IgE antibodies, skin prick tests, and allergic disorders in unselected adolescents. *Allergy* 1981;36:251-6.
- Leitermann K, Ohman JL. Cat allergen I: Biochemical, antigenic, and allergenic properties. *J ALLERGY CLIN IMMUNOL* 1984;74:147-53.
- de Groot H, van Swieten P, van Leeuwen J, Lind P, Aalberse RC. Monoclonal antibodies to the major feline allergen *Fel d I*. Serologic and biologic activity of affinity-purified *Fel d I* and of *Fel d I*-depleted extract. *J ALLERGY CLIN IMMUNOL* 1988;82:778-86.
- Chapman MD, Aalberse RC, Brown MJ, Platts-Mills TAE. Monoclonal antibodies to the major feline allergen *Fel d I*. II. Single-step affinity purification of *Fel d I*, N-terminal sequence analysis, and development of a sensitive two-site immunoassay to assess *Fel d I* exposure. *J Immunol* 1988;140:812-8.
- van der Zee JS, van Swieten P, Jansen HM, Aalberse RC. Skin tests and histamine release with *P₁*-depleted *Dermatophagoides pteronyssinus* body extracts and purified *P₁*. *J ALLERGY CLIN IMMUNOL* 1988;81:884-96.
- Varga JM, Ceska M. Characterization of allergen extracts by PAG-IEF and radioimmunosorbent allergen assay. II. Dog and cat allergens. *Intern Arch Allergy Appl Immunol* 1972;42:438-54.
- Løwenstein H. Allergene von Katze, Hund, Rind und Pferd. *Allergologie* 1981;4:265-9.
- Ford AW, Alterman L, Kemeny DM. The allergens of dog. I. Identification using crossed radioimmuno-electrophoresis. *Clin Exp Allergy* 1989;19:183-90.
- Larsen JN, Ford A, Gjesing B, et al. The collaborative study of the international standard of dog, *Canis domesticus*, hair/dander extract. *J ALLERGY CLIN IMMUNOL* 1988;82:318-30.
- McLean AC, Glovsky M, Hoffman DR, Ghekiere L. Identification of allergens in dog-dander extract. I. Clinical and immunological aspects of allergenicity activity. *Ann Allergy* 1980;45:199-204.
- Brandt R, Yman L. Dog-dander allergens: Specificity studies based on the radioallergosorbent technique. *Intern Arch Allergy Appl Immunol* 1980;61:361-70.
- Viander M, Valovirta E, Vanto T, Koivikko A. Cross-reactivity of cat- and dog-allergen extracts: RAST-inhibition studies with special reference to the allergenic activity in saliva and urine. *Intern Arch Allergy Appl Immunol* 1983;71:252-60.
- Hunter WM, Greenwood FC. Preparation of iodine-131-labeled growth hormone of high specific activity. *Nature* 1962;194:495.
- Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975;256:495-7.
- Kohler G, Milstein C. Derivation of specific antibody-producing tissue culture and tumour lines by cell fusion. *Eur J Immunol* 1976;6:511-9.
- Astaldi GCB, Jansen MC, Lansdorp PM, Willems C, Zeijlmeier WP, Oosterhof F. Human endothelial culture supernatant (HECS): a growth factor for hybridoma. *J Immunol* 1980;125:1411-4.
- Calkhoven PG, Aalbers M, Koshte VL, Pos O, Oei HD, Aalberse RC. Cross-reactivity among birch pollen, vegetables, and fruits as detected by IgE antibodies is due to at least three distinct cross-reactive structures. *Allergy* 1987;42:382-90.
- Pierce J, Suelter CH. An evaluation of Coomassie brilliant blue G-250 dye-binding method for quantitative protein determination. *Anal Biochem* 1977;81:478-80.
- Aalberse RC, Koshte VL, Clemens JGJ. Immunoglobulin E antibodies that cross-react with vegetable foods, pollen, and Hymenoptera venoms. *J ALLERGY CLIN IMMUNOL* 1981;68:356-64.
- Aalberse RC, Van Zoonen M, Clemens JGJ, Winkel IN. The use of hapten-modified allergens instead of solid-phase-coupled antigens in a RAST-type assay. *J Immunol Methods* 1986;87:51-7.
- Schou C, Løwenstein H. Purification and characterization of the important dog allergen *Can f I* (Ag 13) [Abstract]. *J ALLERGY CLIN IMMUNOL* 1990;85:170.
- Lichtenstein LM, Osler AG. Histamine release from human leukocytes by ragweed-pollen antigen. *J Exp Med* 1964;120:507-30.
- Siraganian RP. An automated continuous flow system for the extraction and fluorometric analysis of histamine. *Anal Biochem* 1974;57:383-94.
- Siraganian RP. Refinements in the automated fluorometric histamine analysis system. *J Immunol Methods* 1975;7:283-90.
- Ohman JL, Bloch KJ, Kendall S, Lowell FC. Allergens of mammalian origin. IV. Evidence for common allergens in cat and dog serum. *J ALLERGY CLIN IMMUNOL* 1975;57:560-8.
- Platts-Mills TAE, de Weck AL. Dust mite allergens and asthma—a world-wide problem. *J ALLERGY CLIN IMMUNOL* 1989;83:416-27.
- Luczynska CM, Li Y, Chapman MD, Platts-Mills TAE. Airborne concentrations and particle-size distribution of allergen derived from domestic cats (*Felis domesticus*): measurements using cascade impactor, liquid impinger, and a two-site monoclonal antibody assay for *Fel d I*. *Am Rev Respir Dis* 1990;141:361-7.