

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* Exposure¹

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Two mAb were used to develop new techniques for the purification and quantitation of the major feline salivary allergen, *Felis domesticus* allergen I (*Fel d I*). The allergen was purified from aqueous house dust extract with a high *Fel d I* content by affinity chromatography over a monoclonal immunosorbent and elution with 4 mM HCl, pH 2.5. This single step procedure gave 40 to 50% recovery of 90% pure allergen which, following final purification by size exclusion HPLC, showed a single line on immunodiffusion and crossed immunoelectrophoresis against monospecific anti-*Fel d I* and polyclonal anti-cat dander antibodies. The m.w. of native *Fel d I* was 39,000 on size exclusion HPLC, and 17,000 under nonreducing conditions on gel electrophoresis. The N-terminal amino acid sequence (33 residues) showed no homology with other known protein sequences. The combination of the SDS-PAGE and N-terminal sequence data suggests that *Fel d I* is a non-covalently linked homodimer. A two-site RIA was developed using mAb directed against different epitopes on *Fel d I*. This assay was species-specific, highly sensitive (0.0004 U/ml), and showed an excellent correlation with a polyclonal inhibition RIA ($n = 27$, $r = 0.93$, $p < 0.001$). Cat allergen extracts used for immediate skin tests showed marked differences in *Fel d I* content (from 0.1 to 30 U/ml). Consistently high *Fel d I* levels were found at monthly intervals in six dust samples from four houses with cats (10 to 100 U/g of dust). Comparisons of *Fel d I* and mite and pollen allergen levels showed that house dust can contain $>100 \mu\text{g/g}$ of either of these allergens and is a potent source of foreign environmental antigens. Monoclonal affinity chromatography provides a major breakthrough in the purification of *Fel d I*, from a source material that would otherwise have been considered impossible (house dust). The mAb assay

for *Fel d I* is both more sensitive and more easily standardized than existing techniques. These techniques will allow full structural and antigenic analysis of *Fel d I* and more detailed studies on the relationship between cat antigen exposure and the development of asthma.

Immediate hypersensitivity to inhaled allergen produced by the domestic cat (*Felis domesticus*) has been demonstrated in 20 to 30% of patients with asthma (1–3). Positive immediate skin tests and serum IgG and IgE antibody responses to a single major feline allergen, *Felis domesticus* allergen I (*Fel d I*,³ formerly known as Cat-1), have been demonstrated in most cat-allergic patients (4–6). *Fel d I* is a ~36,000 m.w. acidic protein dimer which was purified and characterized by Ohman and colleagues and has subsequently been identified by other workers using serologic techniques (6–13). It has been used as an immunologic marker to monitor changes in humoral immune response after desensitization treatment and to assess environmental cat antigen exposure (5, 14–17). However, although *Fel d I* is a potent immunogen and an important cause of allergic disease, it has been difficult to purify, and constraints in the availability of purified *Fel d I* have limited further molecular and immunologic studies of this allergen.

Most cat allergen extracts are produced by commercial manufacturers and are not available in large volumes for research purposes. *Fel d I* was originally purified from cat pelt extracts, but these extracts contain many other cat serum proteins and the allergen yield is poor. It is now known that the main source of *Fel d I* is saliva (10, 11, 13, 18). Surface washes of living cats have been used to produce *Fel d I*-enriched extracts (19) and an improved purification scheme using polyclonal affinity chromatography has been reported (9). However, although this technique gives good recovery (~40%), it requires initial affinity purification of large amounts of polyclonal antiserum on *Fel d I* immunosorbents and the reported final yield was low, ~100 units, i.e., $<500 \mu\text{g}$ (9).

The present paper describes a single step procedure for the purification of *Fel d I* from crude house dust extract with a high *Fel d I* content (50 U/ml) using mAb affinity chromatography. We determined the amino acid composition and partial amino acid sequence of the monoclonal

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³ Abbreviations used in this paper: *Fel d I*, *Felis domesticus* allergen I; Fd1A and Fd1B, two *Fel d I*-specific mAb directed against different epitopes; CIE, crossed immunoelectrophoresis.

purified *Fel d 1* and this technique provides sufficient pure allergen (milligram amounts) to proceed with full sequence analysis. In addition, the mAb have been used to develop a simple, highly sensitive and specific, two-site RIA for measuring *Fel d 1* which is suitable for immunologic monitoring of environmental cat antigen exposure.

MATERIALS AND METHODS

mAb. The two *Fel d 1*-specific mAb used in these studies were derived from a single fusion using SP₂/0 myeloma cells and spleen cells from BALB/c which had been immunized with 10% cat dander extract (Haarlems Allergen Laboratorium, Haarlem, the Netherlands) in CFA. These mAb, CLB-Fd1A (Fd1A) and CLB-Fd1B (Fd1B), were produced in the Central Laboratory of the Netherlands Bloodtransfusion Service (CLB); both were isotype IgG1 and were directed against different epitopes on *Fel d 1*. Full details of their production and characterization will be published elsewhere.⁴ Ascites from each clone was produced by injecting 2×10^6 cells i.p. into pristane-primed BALB/c mice and withdrawing the fluid at 10–14 days. The mAb were partially purified from each ascites by precipitation with 50% saturated ammonium sulfate to obtain IgG-rich fractions. Fd1A was further purified by preparative IEF (20, 21) to obtain pure mAb which could be radiolabeled. Purified Fd1A (pI 7.0) was used as second antibody in the monoclonal RIA for *Fel d 1*.

Monoclonal affinity purification of *Fel d 1* from house dust extract. A single house dust sample (WB) was used for allergen purification. This dust came from a house with four cats and was selected after screening several house dusts for *Fel d 1* content. Unsieved WB dust (170 g) from a vacuum cleaner bag was extracted in 1700 ml PBS/0.05% Tween 20/0.5% phenol, overnight at 4°C. The liquid was centrifuged ($10,000 \times g$ 50 min), filtered through Whatman 3 MM chromatography paper and stored at 4°C prior to immunoadsorption.

The monoclonal anti-*Fel d 1* immunosorbent was prepared by coupling 200 mg of a 50% saturated ammonium sulfate fraction of Fd1A ascites to 7 g CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ) in 0.1 M NaHCO₃/0.5 M NaCl, pH 8.3, overnight at 4°C. Based on SDS-PAGE analyses and the preparative IEF results, we estimate that Fd1A mAb comprised 50 to 70% of the protein in the ammonium sulfate fraction of ascites and that the quantity of mAb coupled to Sepharose was ~100 mg. The Fd1A Sepharose (20 ml) was washed successively in 0.1 M acetate/0.5 M NaCl, pH 4.0, followed by 0.1 M Tris HCl/0.5 M NaCl, pH 8.0, (three washes of 200 ml of each buffer), packed into a 2- $\times$ -6-cm column (20-ml plastic syringe barrel) and equilibrated in PBS, pH 7.4. Approximately 100 ml of house dust extract containing 50 U/ml *Fel d 1* was adsorbed over the column at a flow rate of 15 ml/h (all column procedures were carried out at room temperature). The column was washed with 450 ml PBS and then eluted with 4 mM HCl, pH 2.5. Fifty 3-ml fractions were collected at a flow rate of 15 ml/h and pooled on the basis of protein content (absorbance at 280 nm) and *Fel d 1* content (determined by monoclonal RIA). A biphasic elution profile was obtained with two *Fel d 1* peaks at fractions 12 to 18 and 26 to 32. The second peak (fractions 26 to 32) was usually of greater purity. The *Fel d 1* pools were lyophilized, redissolved in 1 to 2 ml of deionized water and stored frozen. Typically, application of 100 ml of WB dust extract containing 5000 U *Fel d 1*, yielded a total of 2000 to 2500 U (~10 mg) purified allergen (i.e., 40 to 50% recovery). This single step adsorption and elution procedure appeared to be the most efficient method of affinity purification. Batch adsorption techniques were used initially, but were abandoned because allergen recovery was <10%.

SDS-PAGE. Allergen preparations (20 to 50 μ g protein) were boiled for 2 min in 0.05 M Tris/4% SDS, pH 6.8, and analyzed by SDS-PAGE in a discontinuous buffer system using a 5% stacking gel and a 20% resolving gel. The gel was electrophoresed at 30 mA for 4 h in 0.025 M Tris/0.19 M glycine/0.1% SDS, pH 8.3, and stained with Coomassie blue R-250.

HPLC. Samples were analyzed by size exclusion on a Spherogel TSK 2000SW column (7.5 $\times$ 600 mm, Altek, Berkeley, CA) using an LKB 2150 HPLC system (LKB Instruments, Gaithersburg, MD). The column was calibrated with OVA (m.w. 43,000), chymotrypsinogen (m.w. 27,000), and cytochrome c (m.w. 12,500). *Fel d 1* preparations were analyzed isocratically by injecting 20- μ l samples (10 to 20 U allergen) onto the column and eluting in 0.1 M phosphate buffer/0.2 M NaCl, pH 6.8, at a flow rate of 0.5 ml/min (total run time = 55 min). For preparative purposes, 100 to 200 U *Fel d 1* was applied in a volume of

0.2 ml. Fractions of 1 ml were collected and pooled, and the *Fel d 1* peak was dialyzed against deionized water and lyophilized.

Crossed immunoelectrophoresis. Allergen preparations were analyzed by crossed immunoelectrophoresis (CIE) with intermediate gel using polyclonal rabbit anti-cat dander antibodies (Batch 524181, Allergologisk Laboratorium, Copenhagen, Denmark). Agarose gels (1%) were cast on 8 $\times$ 9 cm glass plates and electrophoresis was carried out on an LKB Multiphor. Allergen extracts (10 μ l), containing 20 to 50 U *Fel d 1*/ml, were electrophoresed in 0.1 M Tris-barbital buffer, pH 8.6, at 150 V $\times$ 20 mA for 2 h (first dimension) and at 120 V $\times$ 16 mA for 16 h (second dimension). The second dimension gel (10 ml) contained 0.1 to 0.2 ml rabbit anti-cat antibodies. Gels were pressed dry and stained with Coomassie blue R250.

N-terminal sequence analysis. A total of 50 U *Fel d 1* was sequenced on an Applied Biosystems 420-A protein sequenator and PTH amino acids were identified on an Applied Biosystems 120-A liquid chromatograph (Applied Biosystems, Foster City, CA).

Monoclonal RIA for *Fel d 1*. A 50% saturated ammonium sulfate fraction of anti-*Fel d 1* mAb, Fd1B, was coupled to CNBr-activated paper discs (10 μ g Fd1B/disc) in 0.1 M bicarbonate buffer containing 0.5 M NaCl, pH 8.4, overnight at 4°C. The discs were washed twice in 0.2 M glycine, pH 8.0, four times in PBS/0.05% Tween 20 containing 1% BSA (BSA PBS-T, assay diluent) and then placed in flat-bottomed polystyrene microtiter plates. A diluted allergen sample (0.1 ml) was incubated with each disc for 4 h. The disc was washed five times with PBS-T and incubated overnight with 5 ng [¹²⁵I]-labeled IEF purified, monoclonal anti-*Fel d 1* (Fd1A, sp. act. 30 μ Ci/ μ g). After a further wash cycle, the disc was dried and counted in a gamma counter. The quantity of Fd1A bound was directly related to the quantity of *Fel d 1* in the sample and values were obtained from a control curve using cat reference E3 (Office of Biologics and Research Resources, Bethesda, MD). This cat dander extract is the Office of Biologics and Research Resources standard for radial immunodiffusion and contains 10.5 U/ml *Fel d 1*. Doubling dilutions of cat E3 were used to form a control curve, from 52.5 to 0.4 mU *Fel d 1*/ml, which was run in parallel with each assay. All samples were assayed at four different dilutions and values were interpolated from the control curve.

Polyclonal inhibition RIA. Diluted allergen samples (0.05 ml) were incubated with 0.05 ml of a 1/5000 dilution of polyclonal rabbit anti-cat dander antibodies for 2 h in microtiter wells. Aliquots of 5 ng [¹²⁵I]-labeled *Fel d 1* (sp. act. 20 to 40 μ Ci/ μ g) were added to each well, together with a goat anti-rabbit IgG coupled paper disc (1 μ l antiserum/CNBr-activated disc) and incubated overnight. The discs were washed five times with PBS-T, dried, and counted as above. The quantity of [¹²⁵I] *Fel d 1* bound was inversely related to the quantity of allergen in the sample and values were obtained using a control curve of cat reference E3.

RESULTS

Monoclonal affinity purification of *Fel d 1*. A 10% aqueous house dust extract (WB) containing 50 U/ml *Fel d 1* was passed over a monoclonal anti-*Fel d 1* immunosorbent column and, after extensive washing, bound allergen was eluted in 4 mM HCl, pH 2.5. The capacity of the column, as judged by its ability to remove *Fel d 1* from the dust extract, was 5,000 to 10,000 units and 40 to 50% of the applied allergen (i.e., up to 5,000 units) was usually recovered. The single step purified allergen was >90% homogeneous on SDS-PAGE. WB dust extract showed only one protein band (at 60 kDa) under nonreducing conditions on SDS-PAGE, whereas affinity-purified *Fel d 1* showed a dense band at 17 kDa and three faint bands at 11 to 14 kDa (Fig. 1). The 17 kDa *Fel d 1* band was diffuse, however, the same staining pattern was seen using several different allergen preparations and by applying smaller quantities of the sample, suggesting that this is an intrinsic property of the protein. On size exclusion HPLC under native conditions, the *Fel d 1* eluted as a major peak of 39 kDa, when compared with the mite allergen, *Der f 1*, which eluted at 18 kDa (Fig. 2). The single step monoclonal purified *Fel d 1* showed a light brown color, probably due to nonspecific retention of non-dialyzable pigments on the affinity column. Rechromatography of these preparations either on HPLC or over the monoclonal immunosorbent, resulted in a single, colorless peak of purified allergen.

⁴ De Groot, H., P. van Swieten, J. van Leeuwen, P. Lind, and R. C. Aalberse. Monoclonal antibodies to the major feline allergen *Fel d 1*. I. Biologic activity of affinity-purified *Fel d 1* and of *Fel d 1*-depleted extracts. Submitted for publication.

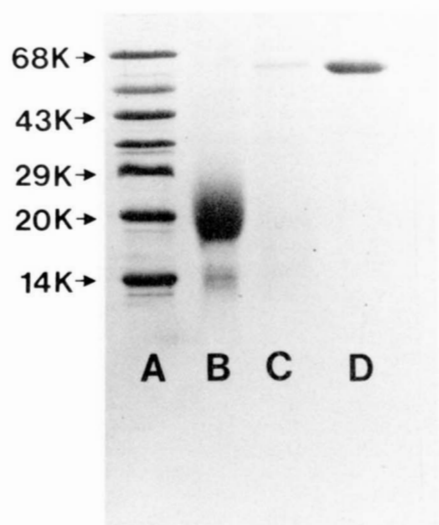


Figure 1. SDS-PAGE analysis of monoclonal affinity-purified *Fel d 1*. Samples were analyzed under nonreducing conditions on a 20% resolving gel with a 5% stacking gel as follows: lane A, m.w. markers; lane B, single step affinity-purified *Fel d 1* (50 U); lane C, WB house dust extract pre-absorption on Fd1A mAb immunosorbent; lane D, WB dust extract post-absorption on Fd1A column (30- μ l samples of each dust extract).

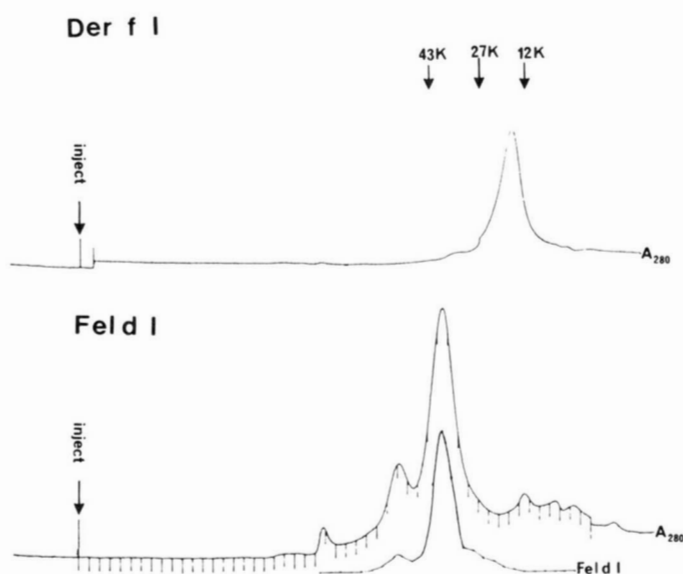


Figure 2. Comparison of affinity-purified *Fel d 1* and *Der p 1* on size exclusion HPLC. *Fel d 1* and *Der p 1* were purified by monoclonal affinity chromatography on Fd1A and 4C1 B8 immunosorbents, respectively, and then analyzed on a Spherogel TSK 2000SW column. Almost all the *Fel d 1* (6 U/ml, as determined by mAb RIA) eluted in parallel with a large protein peak, m.w. 39,000. Homogeneous *Fel d 1* was obtained by rechromatography of this peak over the same column. *Der f 1* eluted as a single peak, m.w. 18,000.

The immunochemical purity of *Fel d 1* purified by sequential monoclonal affinity chromatography and HPLC was demonstrated by immunodiffusion and CIE (Fig. 3). The allergen showed a single line of identity against polyclonal rabbit anti-cat dander antibodies and monospecific sheep anti-*Fel d 1* (kindly provided by Dr. John Ohman, Veteran's Administration, Boston, MA) (Fig. 3A) and also showed a single line against the polyclonal antibodies on CIE (Fig. 3C). CIE analysis of WB dust demonstrated the presence of five different cat antigens (Fig. 3B). The *Fel d 1* precipitin arc on the CIE plates was significantly retarded when both anti-*Fel d 1* mAb (Fd1A and Fd1B) were incorporated into the intermediate gel (Fig. 3D and E).

The amino acid composition and sequence of 33 N-terminal amino acids of *Fel d 1* was determined (Table I and Fig. 4). The N-terminal sequence analysis was initially carried out using the same *Fel d 1* preparation as shown in Figure 1 and was subsequently confirmed (out to residue 21) using fully purified allergen (Fig. 3C). Sequence similarity searches of the National Biomedical Research Foundation protein sequence data base using the computer algorithm FASTP (22) showed no homology between the *Fel d 1* N terminus and other known protein sequences.

Monoclonal RIA for *Fel d 1*. A two-site immunoassay for the quantitation of *Fel d 1* was developed using mAb Fd1B coupled to cellulose discs and ^{125} I-labeled, IEF-purified Fd1A to detect bound allergen. This assay could detect as little as 0.0004 U (i.e., ~ 2 ng) *Fel d 1* and showed an excellent quantitative correlation when compared with an inhibition RIA using polyclonal anti-cat antibodies and ^{125} I-labeled *Fel d 1* ($n = 27$, $r = 0.93$, $p < 0.001$; Fig. 5). Significant quantities of *Fel d 1* were detected in cat saliva (4.75 U/ml), but none was detected in urine (< 0.0004 U/ml) and the levels in serum were very low (< 0.01 U/ml). *Fel d 1* levels were compared in a panel of diagnostic allergen extracts and the assay specificity was assessed using epithelium extracts from several different animal species (Table II). Commercial cat epithelium extracts varied by 300-fold in *Fel d 1* content (from 0.1 to 30 U/ml). Furthermore, five cat extracts contained less *Fel d 1* than one of the house dust extracts (D, Table II) and even the most potent extract (G, Table II) contained almost twofold less *Fel d 1* than the WB house dust extract used as source material for allergen purification. Only two of 15 animal epithelium extracts (dog and horse) showed detectable *Fel d 1*. Although this could represent minor cross-reactivity, it is more likely to indicate contamination of these extracts with trace amounts of cat allergens, because other dog and horse epithelium extracts did not contain *Fel d 1*.

The mAb RIA was used to compare *Fel d 1* levels in dust samples collected year round (from February 1984 to 1985) in the Charlottesville area (23). In six samples from four houses with cats, *Fel d 1* levels were very high (10 to 100 U/g of dust), whereas in five houses without cats, *Fel d 1* levels were low (< 1 U/g) but readily detectable (Fig. 6). There was no evidence for seasonal variation in *Fel d 1* levels and, by comparison with previous studies, no correlation with either the mite or grass allergen levels in these dust extracts (23). Selected examples illustrating the range and distribution of both *Fel d 1* and other foreign antigens present in house dust are shown in Table III.

DISCUSSION

It has been known for many years that house dust contains a variety of inhalant allergens (mites, animal danders, insects, fungi, etc.), however, dust extracts have previously been regarded as very poor source material for purification purposes because of their low potency and difficulties in accurately assessing their allergen content. The purification of major indoor allergens, e.g., *Fel d 1*, *Der p 1*, *Der f 1*, and the development of allergen-specific mAb now provide much better methods for analyzing allergen levels in dust extracts (24) and for identifying extracts which could be used in purification schemes. The results reported here demonstrate the first purification of a major allergen direct from house dust, by mAb affinity chromatography using a carefully selected house dust extract. This is a simple, sin-

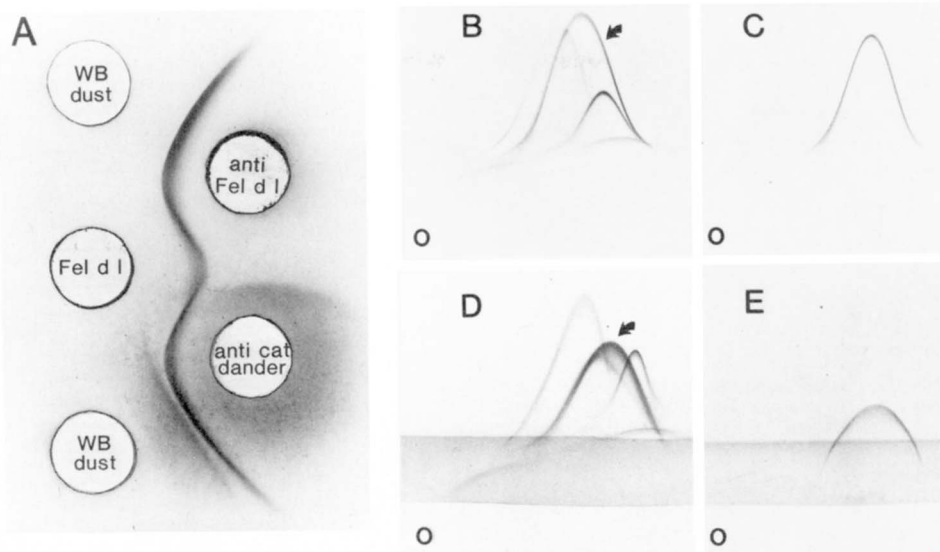


Figure 3. Antigenic analysis of WB house dust extract and *Fel d 1*. The samples were analyzed as follows: A, immunodiffusion using polyclonal rabbit anti-cat dander antibodies and monospecific sheep anti-*Fel d 1*; B and C, WB dust (B) and *Fel d 1* (C) were analyzed by CIE using rabbit anti-cat dander antibodies; D and E, a 1/10 dilution of Fd1A and Fd1B ascites was incorporated into the intermediate CIE gel, prior to second dimension electrophoresis into rabbit anti-cat dander antibodies. In these analyses, the *Fel d 1* precipitin arc was significantly retarded by the mAb when both WB dust (D) and *Fel d 1* (E) were compared. The *Fel d 1* precipitin arc in WB dust extract is indicated by the arrows in B and D. Sample wells are indicated (o).

TABLE I
Amino acid composition of *Fel d 1*^a

Amino acid	Residues/100 residues
Aspartate	10.8
Glutamate	13.5
Cysteine	ND
Serine	9.2
Glycine	6.3
Histidine	0.0
Threonine	7.2
Alanine	8.5
Arginine	3.1
Proline	4.3
Tyrosine	2.9
Valine	8.0
Methionine	2.5
Isoleucine	3.8
Leucine	10.5
Phenylalanine	2.9
Lysine	6.6

^a Samples of *Fel d 1* (10 μ l at 220 U/ml) were hydrolyzed in vacuo in 6 N HCl, overnight, and then analyzed in triplicate on a Waters model 840 HPLC system. Results are the mean values obtained using the same preparation of tandem monoclonal and HPLC purified *Fel d 1*. Cysteine was not determined (ND).

NH₂-Glu - Ile - Thr - Pro - Ala - Val - Lys - Arg - Asp - Val¹⁰
(Met)
Asp - Leu - Phe - Leu - Thr - Gly - Thr - Pro - Asp - Glu²⁰
Tyr - Val - Glu - Gln - Val - Ala - Gln - Tyr - (Lys) - Ala³⁰
Pro - Asp - Val³³
(Val)

Figure 4. N-terminal amino acid sequence analysis of *Fel d 1*. Identification of three of the amino acids was questionable and residues shown in parentheses could substitute at those positions.

gle step procedure for purifying *Fel d 1* from aqueous, non-dialyzed house dust extract which is highly efficient (>200-fold purification) and yields 5 to 20 mg *Fel d 1*. It has not previously been possible to obtain such high yields using either conventional biochemical separation techniques or polyclonal affinity chromatography. The success of the mAb purification technique is related to two factors. First, several hundred milligrams of the mAb can be readily produced and used to prepare high capacity immunosorbents. Second, the house dust extract used as source material con-

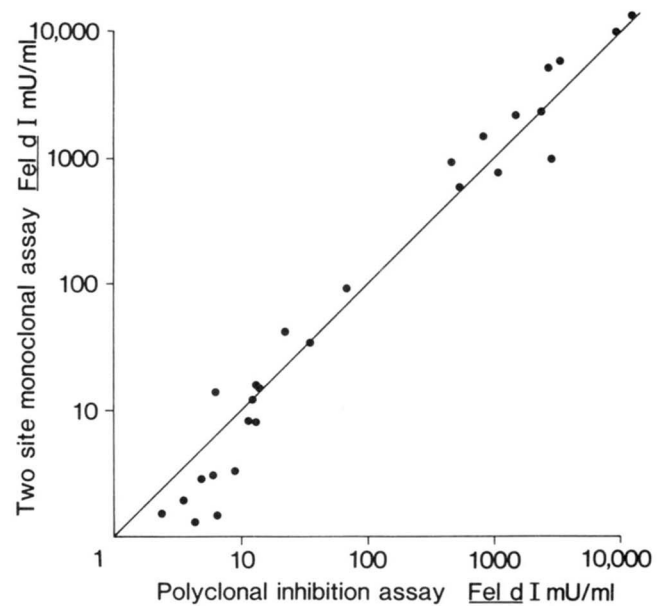


Figure 5. Correlation between the monoclonal RIA and a polyclonal inhibition RIA for *Fel d 1*. Of 27 house dust samples previously collected in Charlottesville, VA, 23 were compared in each assay and the results expressed in U *Fel d 1*/g of dust. Linear regression analysis showed a very good quantitative correlation between the two assays ($r = 0.93$, $p < 0.001$).

tains a high concentration of *Fel d 1* (100 to 200 μ g/ml), relative to other proteins or cat antigens (as judged by SDS-PAGE and CIE analysis). The WB dust extract contained approximately 100-fold lower levels of mite allergen (107 ng/ml *Der p 1* and 299 ng/ml *Der f 1*) and appeared to be selectively enriched for *Fel d 1*. However, it is still a poor source material when compared, for example, with pollen extracts, which may contain several mg/ml *Lol p 1* or *Amb a 1*, and it would be very difficult to isolate *Fel d 1* from this extract without mAb affinity chromatography. The availability of milligram amounts of *Fel d 1* enabled us to obtain absolute weight estimates, based on amino acid composition and the dry weight of a lyophilized 2-mg sample. Those estimates were in good agreement (Table III) and suggest that 1 U *Fel d 1* represents 4 μ g protein. Although *Fel d 1*

TABLE II
Two-site monoclonal RIA for *Fel d 1*: specificity and comparison of
Fel d 1 levels in commercial allergen extracts^a

Manufacturer ^b	<i>Fel d 1</i> concentration (U/ml) in		
	Cat	Dog	House dust
A	28.40	NA	NA
B	2.80	0.01	<0.0004
C	0.63	<0.0004	0.52
D	0.73	<0.0004	2.63
E (i)	1.41	<0.0004	0.40
E (ii)	15.90	NA	NA
F	0.28	<0.0004	0.70
G	30.90	<0.0004	0.10
H	0.12	<0.0004	0.09

^a A panel of animal epithelium extracts and house dust extracts from eight commercial suppliers was assayed for *Fel d 1* by mAb RIA and the results were quantitated using the cat reference E3 (see *Materials and Methods*). Extracts of mouse, rat, rabbit, guinea pig, gerbil, cow, and goat epithelia contained no detectable *Fel d 1* and a horse hair extract was weakly positive (0.009 U/ml).

^b Cat, dog, and house dust extracts were obtained from seven U.S. and one U.K. manufacturer. These extracts were prepared either on a 1/10 to 1/50 w/v basis or as solutions containing 10,000 to 40,000 protein nitrogen U/ml. Extracts A and E (ii) were "standardized" cat extracts and comparable dog and house dust extracts were not available (NA).

TABLE III
Concentration of *Fel d 1* and other foreign antigens in selected house
dust samples^a

Sample	<i>Fel d 1</i> (μ g/g)	<i>Der p 1</i> (μ g/g)	<i>Der f 1</i> (μ g/g)	<i>Lol p 1</i> (μ g/g)
1	472.0	<0.2	<0.2	<0.2
2	<0.2	1.7	240.0	<0.2
3	20.6	26.5	0.4	1.9
4	<0.2	0.8	0.2	1.3
5	2.2	1.9	0.2	148.3
6	6.7	20.5	9.3	33.4

^a Samples were analyzed for *Fel d 1*, *Der p 1*, and *Der f 1* by mAb RIA (24) and for *Lol p 1* using a previously described polyclonal capture RIA (23). Samples 1 and 2 were collected in a previous study carried out in Charlottesville, VA (23); the other four were from a recent study carried out at Travis Air Force Base, CA. Absolute levels for *Fel d 1* were calculated assuming 1 U *Fel d 1* = 4 μ g. This estimate was based on the amino acid composition. On a dry weight basis, 2 mg of a saltfree preparation of *Fel d 1* ($A_{280} = 1.3$) contained 402 U/ml, giving a value of 5 μ g/U. *Der p 1* and *Der f 1* values were calculated relative to the International Union of Immunological Societies *D. pteronyssinus* Reference (code NIBSC 82/518) which contains 12.5 μ g/ml *Der p 1*. Values for *Lol p 1* were calculated assuming 1 U of rye grass pollen allergen contains 0.1 ng *Lol p 1* (23).

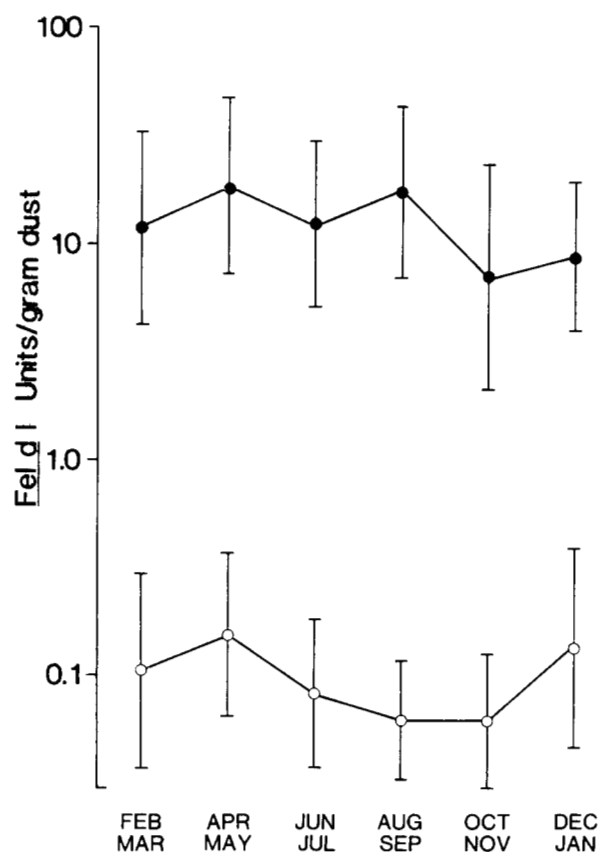


Figure 6. Year-round analysis of *Fel d 1* levels in house dust samples. Of the 23 samples from the Charlottesville year-round study (23), six were extracts from four houses with cats and five were extracts from houses without cats. Data are plotted as the geometric mean $\pm$ 95% confidence limits for cat-positive (●) and cat-negative (○) houses.

was first isolated over 10 yr ago by Ohman (7–9), it has only been successfully purified in one other laboratory (6). The mAb described here are available for research purposes and screening dust extracts for *Fel d 1* content by mAb immunoassay is straightforward. This purification technique should dramatically improve the availability of *Fel d 1* and will have important applications in research studies which require large quantities (milligrams) of allergen, e.g., protein sequence analysis, antigenic structure, and T cell

responses.

Previous work by Ohman and colleagues showed that *Fel d 1* is a 35,000 m.w. dimer comprised of two noncovalently linked 18,000 m.w. subunits, which occurs in three isoallergenic forms (pI 3.5 to 4.1) (7–9). The preliminary structural data on *Fel d 1* reported here is consistent with those studies. Our N-terminal amino acid sequence analysis was carried out twice on nonreduced *Fel d 1* preparations and on each occasion the same sequence was obtained, suggesting that *Fel d 1* is a homodimer. Recent thermal denaturation and reduction and alkylation experiments suggest that dissociated *Fel d 1* subunits retain their antigenicity and that some of the antigenic sites on *Fel d 1* are dependent on amino acid sequence (9). Interestingly, we found no homology between the *Fel d 1* partial sequence and other N-terminal sequences in the protein data base, thus raising the fascinating possibility that this protein, whose function is currently unknown, is unique to the domestic cat. Full sequence analysis will now be possible and will clearly be essential for further investigating the antigenic structure of this molecule and possibly its function. The mAb will also be useful for defining the repertoire of antigenic sites on *Fel d 1*. The use of Fd1A and Fd1B in a two-site assay for *Fel d 1* suggests that they are directed against nonoverlapping epitopes; a finding which has recently been confirmed in epitope mapping studies using ¹²⁵I-labeled mAb. We have also recently produced a panel of 11 mAb to *Fel d 1* and epitope mapping studies using these mAb, in addition to Fd1A and Fd1B, define three nonoverlapping epitopes and one partially overlapped epitope on *Fel d 1* (data not shown).

Feline allergens have previously been measured using several different techniques, including single radial immunodiffusion, rocket electrophoresis, leukocyte histamine release, and radioallergosorbent test inhibition (6, 9, 10, 16, 25). These techniques are difficult to standardize because they require monospecific precipitating animal antisera or human reagents. In contrast, the mAb assay for *Fel d 1* combines the consistent specificity of the mAb (which can be maintained for many years) together with economical use of cat allergen references (e.g., cat E3 or the WHO cat reference which is currently in preparation). The mAb assay avoids the need to produce precipitating

antisera (although ironically this will now be much easier using mAb purified *Fel d I*), is approximately 100-fold more sensitive than immunodiffusion-based techniques and should be very useful for the precise quantitation of *Fel d I* in allergen extracts. The need for such an assay is emphasized by the present results which demonstrate striking differences in the *Fel d I* content of cat allergen extracts that are currently used to demonstrate IgE-mediated reactions by skin testing. In many of these extracts, the quantities of *Fel d I* were too low to be measured accurately using immunodiffusion based techniques. Our results suggest that measurements of *Fel d I* will significantly improve the standardization of cat allergen extracts and will allow further evaluation of the doses of *Fel d I* required for successful immunotherapy (14, 26).

Previous studies suggest that allergic individuals become sensitized after inhalation of extremely low doses of aero-allergens ($<1 \mu\text{g/yr}$ of major mite or pollen allergens) and that the ability to recognize and respond to these minute antigen doses (with no apparent adjuvant) is important in the development of immediate hypersensitivity (27, 28). Studies on indoor allergen exposure have focused on the quantities of allergen present in dust and the quantities and size of allergen particles that become airborne and could be inhaled and deposited in the respiratory mucosa (16, 17, 23–25, 29–31). The present results confirm recent observations that very high levels of *Fel d I* can accumulate in house dust (32, 33). They also show that the quantities of major inhalant allergens in house dust can vary enormously, from $<0.1 \mu\text{g/g}$ to $>100 \mu\text{g/g}$ dust, and that individual dust samples may show striking differences (>100 -fold) in their content of any one allergen (as illustrated in Table III). We believe that inhalant allergens can comprise a significant amount of the foreign antigenic protein in house dust and may also be among the most common antigens to which humans are naturally exposed (indeed, in some dust extracts, including samples 1 and 2 (Table III), they can account for up to 10 to 20% of the soluble protein). This would explain why IgE antibody responses to these indoor allergens are extremely common, i.e., in $\sim 20\%$ of the population. The *Fel d I* levels reported here are comparable to the levels of mite allergens that are associated with allergic symptoms ($>10 \mu\text{g/g}$) (23) and suggest that immunologic monitoring of allergen exposure using mAb assays, together with allergen avoidance procedures, could significantly improve clinical management. Clearly, the development of allergic disease is related to the quantities and form of the allergens that become airborne, as well as to genetic and immunoregulatory factors which influence the development of immediate hypersensitivity. The sensitivity of mAb assays is ideal for detecting nanogram quantities of airborne allergens and these assays have proved most effective in preliminary particle sizing experiments on *Fel d I* (data not shown).

In conclusion, the application of mAb has dramatically changed our ability to study the major feline allergen, *Fel d I*. These changes will make possible full structural analysis of this protein and further understanding of the human immune response and of the relationship between allergen exposure and allergic disease.

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