

Monoclonal antibodies to the major feline allergen *Fel d I*

I. Serologic and biologic activity of affinity-purified *Fel d I* and of *Fel d I*-depleted extract

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Monoclonal antibodies were raised against a major feline allergen, Fel d I. The specificity of the antibodies for Fel d I was demonstrated by a modified crossed immunoelectrophoretic procedure. These antibodies were used in affinity purification and depletion. Fel d I was eluted from the affinity matrix at a low pH. It was tested in serologic and biologic assays. Results of both the RAST and the histamine release test with affinity-purified Fel d I confirmed that it is a very potent allergen for the majority of patients allergic to cats. In the extract depleted with the monoclonal antibody, the ratio Fel d I/cat albumin was reduced by a factor of 20. With this depleted extract, 18 of 25 patients showed a reduction >50% of the bound anti-IgE in the RAST compared with the RAST with crude cat extract. In the histamine release test with cells from two patients, the activity of the depleted extract was reduced by factors of 30 and 40, respectively. Depletion of Fel d I by polyclonal antibodies resulted in a reduction of the ratio Fel d I/cat albumin by a factor of 330. In the RAST, a reduction of the IgE response >50% was found in 23 of 25 patients; in 12 patients the reduction was >90%. In the histamine release test, the polyclonally depleted extract was 200 to 300 times less potent than the crude cat-dander extract. These results indicate that a very large proportion of the allergenic activity of cat-dander extract is caused by a single molecule: Fel d I. (J ALLERGY CLIN IMMUNOL 1988;82:778-86.)

Cats are an important source of indoor allergens. Large quantities of cat allergen are found in houses with cats, but even in houses without cats considerable amounts of cat allergen can sometimes be found.¹ Cat-dander allergy is demonstrable in 20% to 30% of patients with asthma.²⁻⁵ In our own patient population, the incidence of sensitization to cat dander is close to 30%, compared with an incidence of mite sensitization

of 40%. Therefore, we are interested in investigating the human immune response to the major allergen(s) of the cat, especially in view of studies performed by Leitermann and Ohman⁶ indicating that a single allergen, Cat I (new nomenclature *Fel d I*, identical to Ag4^{7,8}), might be the major allergen.

The term *major allergen* is often used for antigens that cause reactions in the majority of patients.⁹ Because the reaction depends in part on the sensitivity of the assay and on the concentration of the test material used, most investigators would like to include a quantitative criterion. One criterion could be a minimum quantity of IgE antibody or a minimum concentration required for biologic activity.

As an alternative definition of a major allergen, one could use as a criterion the effect of removal of the allergen from the extract, because such removal should significantly diminish the allergenic activity of the extract for most patients. For such depletion studies, monoclonal antibodies are indispensable.

Studies by van der Zee et al.¹⁰ demonstrated that a

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Abbreviations used

PBS:	Phosphate-buffered saline solution
<i>Fel d I</i> :	Proposed name for <i>Felix domesticus</i> allergen 1, or Ag4
<i>Der p I</i> :	Major allergen of house dust mite (<i>Dermatophagoides pteronyssinus</i>), identical to P1 or Ag42
SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
CIE:	Crossed immunoelectrophoresis
BSA:	Bovine serum albumin
PBS-AT:	PBS containing 0.3% (wt/vol) BSA, 0.1% (vol/vol) Tween 20, and 5 mmol/L NaN ₃
HAL:	Haarlems Allergenen Laboratorium (Haarlem, The Netherlands)
ALK:	Allergologisk Laboratorium Kopenhagen (Copenhagen, Denmark)
ARTU:	ARTU Biologicals (Lelystad, The Netherlands)

major allergen of the house dust mite *Dermatophagoides pteronyssinus*, *Der p I*, is very potent when isolated and purified. Nevertheless, only a slight effect of *Der p I* depletion on biologic activity was measured with the histamine release test and the skin test.

In the studies presented here, we investigated the biologic and serologic activity of affinity-purified *Fel d I* and of cat-dander extract from which *Fel d I* was removed.

MATERIAL AND METHODS

Allergens

Cat-dander extracts were obtained from HAL, ALK, and ARTU. These extracts contained 66, 52, and 70 FDA U/ml *Fel d I*, respectively. Cat serum (*Fel d I* content <0.5 U/ml) was obtained from Harlan CPB (T.N.O., Zeist, The Netherlands).

Antisera

Three hyperimmune antisera to cat dander were used: (1) polyspecific rabbit anticat serum obtained from ALK; (2) monospecific antibodies obtained by immunization of two rabbits with affinity-purified *Fel d I*, and (3) monospecific sheep anti-*Fel d I*, used as the reference antiserum, provided by Dr. H. Baer (Bureau of Biologics, Food and Drug Administration, Bethesda, Md.).

Polyclonal goat antimouse immunoglobulin and sheep anti-IgE were obtained from the Department for the Production of Immune Reagents (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands). These antibodies were affinity purified and labeled with ¹²⁵I by the chloramine-T technique.

The labeling was performed as described by Hunter and Greenwood.¹¹

Other reagents

PBS-AT contained PBS (0.01 mol/L phosphate, 0.14 mol/L NaCl) with 0.3 mg/ml BSA (Poviet, Amsterdam, The Netherlands), 10 mmol/L EDTA, and 5 mmol/L NaN₃, and 0.1% (vol/vol) 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 were selected from samples submitted for a routine allergic evaluation. Twenty-five samples were selected for the RAST; these sera were characterized by a RAST score to cat dander (HAL) of >10% bound IgE.

For the histamine release test, cells were obtained from four patients selected for a clearly positive RAST score with cat dander (>10% anti-IgE bound), a positive history of cat allergy, and a strongly positive intracutaneous reaction with cat-dander extract.

Production of monoclonal antibodies

Balb/c mice were immunized subcutaneously with 50 µl of a 10% cat-dander extract (HAL) 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 given 4 days before removal of the spleen. Hybridization was performed as described by Kohler and Milstein,^{12, 13} with some modifications.¹⁴ Antibody-producing hybrids were selected by a RAST with Sepharose-coupled cat-dander extract and ¹²⁵I-labeled goat antimouse IgG. Positive wells were further tested with an indirect RAST system: Sepharose-coupled monoclonal antibodies were incubated with cat-allergen extract, and allergen binding was quantitated with human IgE antibodies and ¹²⁵I-sheep anti-IgE.¹⁵ Cloning was performed by repeated limiting dilution. Anticat ascites were obtained from Pristane-treated Balb/c mice. Two stable clones, producing anti-*Fel d I* antibodies, were obtained and were coded Fd-1a and Fd-1b. A third clone specific for cat albumin is called Fd-3.

Specificity of monoclonal antibodies

The specificity of the monoclonal antibodies for *Fel d I* was established by CIE, as follows. Thirty micrograms of reference cat-dander extract (ka 0580) was applied in the well. This first-dimension gel contained 30 µl of the monoclonal antibody. The anodic gel contained 5.7 µl/cm² of rabbit antibodies to cat-dander extract (ALK, lot no. 22-33). Electrophoretic conditions in the first dimension were 10 V/cm for 30 minutes; in the second dimension electrophoresis was at 2 V/cm for 16 hours in 1% agarose (HSA, Litex) with Tris-barbitone buffer (pH 8.6, ionic strength = 0.02). After pressing, washing, and drying, plates were stained in Coomassie brilliant blue.¹⁶

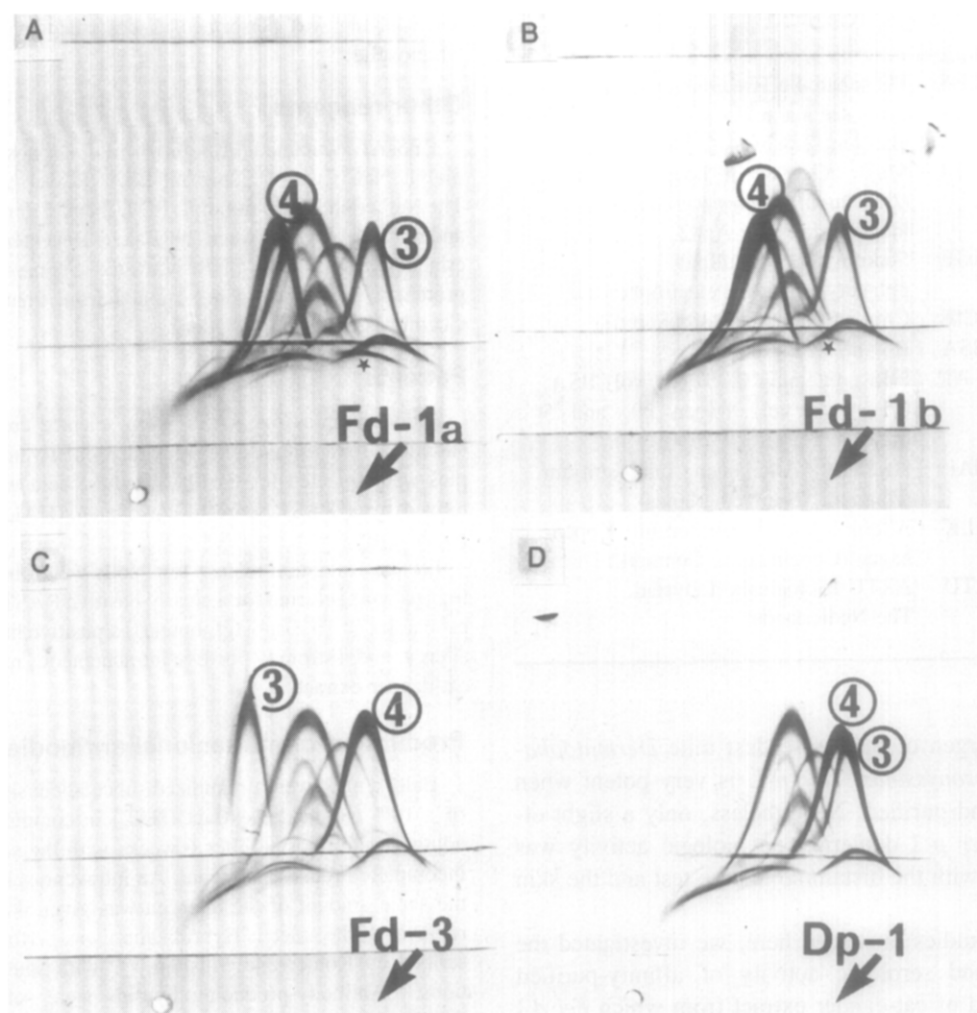


FIG. 1. Analysis of the specificity of the monoclonal antibodies by CIE. Antibody-containing ascites were incorporated in the first dimension gel (30 μ l ascites per plate). The positions of Ag3 (cat serum albumin) and Ag4 (*Fel d* I) are indicated. The position of the variant of *Fel d* I as described in the text is indicated by *. **A-B**, Fd-1a and Fd-1b ascites contain antibodies to *Fel d* I (Ag4). **C**, Fd-3 ascites contain antibodies to cat albumin (Ag3). **D**, Monoclonal antibodies (Dp-1 ascites) against allergen *Der p* II from the house dust mite, used as a control. The pattern in **D** is the same as the reference pattern, except for an increase in the area of the albumin precipitate. This increase is probably due to a partial immunochemical identity between cat albumin and mouse albumin present in mouse ascites.

Affinity purification of *Fel d* I

Affinity purification of *Fel d* I was performed with monoclonal and polyclonal antibodies. As a source of allergen, house-dust extract rich in *Fel d* I was used. The extract was concentrated by absorption on benzoic acid according to the method of Sutherland.¹⁷ An excess of house-dust concentrate (containing 45 U *Fel d* I/ml) was absorbed overnight to Fd-1a-Sepharose (10 ml ascites coupled per gram CNBr-activated Sepharose). *Fel d* I was eluted with glycine HCl (0.065 mol/L, pH 2.4). This material was used for radio-labeling and for the immunization of rabbits. Affinity pu-

rification of *Fel d* I by monoclonal antibodies yielded approximately 14 U *Fel d* I per milliliter of ascites.

The polyclonal antibodies obtained after the immunization of the rabbits were affinity purified and coupled to Sepharose (17 mg/gm CNBr-activated Sepharose) and incubation with an excess of another house-dust extract containing cat dander was performed (dust containing 78 U *Fel d* I per milliliter). The elution of *Fel d* I from the polyclonal antibodies yielded approximately 20 U *Fel d* I per milligram antibody applied. This *Fel d* I was used for the RASTs and the histamine release test.

TABLE I. Concentration of *Fel d I* and cat albumin in extracts used in the RASTs with the patient panel

	<i>Fel d I</i> (U/ml)	Cat albumin (μ g/ml)	Ratio <i>Fel d I</i> / albumin (U/ μ g)
Cat dander (ARTU)	69.0	118.0	0.59
Sham-absorbed extract (2 $\times$)	35.0	56.0	0.63
Sup (monoclonal depletion) (1 $\times$)	0.68	7.5	0.09
Sup (monoclonal depletion) (2 $\times$)	0.24	5.4	0.04
Sup (polyclonal depletion) (1 $\times$)	<0.1	54.0	<0.002
Sup (polyclonal depletion) (2 $\times$)	<0.025	17.0	<0.0015

Labeled antigens

¹²⁵I-*Fel d I*. Allergen eluted from the Fd-1a adsorbent was labeled with the chloramine-T technique (1 mCi added to 10 U *Fel d I*), followed by gel filtration over Sephadex G75. Immune reactivity was usually well >50%. Occasionally, we found a lower immune reactivity after the labeling. When a labeled preparation was bound to and eluted from the Fd-1a adsorbent, immune reactivity >90% was obtained.

¹²⁵I-cat serum albumin. Albumin was isolated from cat serum by gel filtration. Labeling was achieved with 1 mCi iodine 125 for 100 μ g of cat serum albumin.

Fel d I assay

Fifty microliters of an allergen-containing sample was mixed with 50 μ l of a 1:1000 dilution of rabbit antiserum directed against cat dander (ALK). Incubation was performed for 2 hours at room temperature. Then 500 μ l of protein A Sepharose (concentration 2 mg/ml) and 50 μ l of ¹²⁵I-*Fel d I* were added and the suspension was incubated overnight. After centrifugation and washing of the Sepharose, bound radioactivity was measured. *Fel d I* levels are expressed in FDA units, relative to the reference preparation EI-Ct (35 FDA U/ml).

Cat albumin assay

The method for the cat albumin assay was similar to that of *Fel d I*. Fifty microliters of a 1:2000 dilution of rabbit anticat serum was mixed with 50 μ l of a sample. After 2 hours, 500 μ l of protein A Sepharose and 50 μ l of ¹²⁵I-cat albumin were added and incubated overnight. Cat albumin

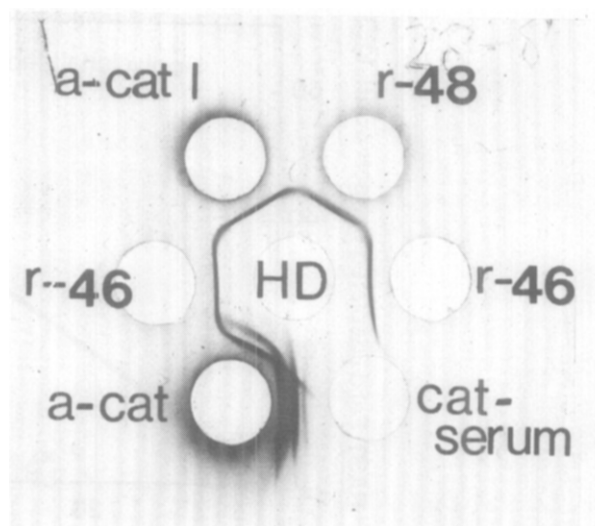


FIG. 2. Specificity of two rabbit antisera r-46 and r-48, obtained by immunization with affinity-purified *Fel d I*. *a-Cat I* is the reference monospecific sheep anti-*Fel d I*; *a-cat* is a polyspecific antiserum to whole cat dander extract; *HD* refers to house-dust extract.

levels are expressed as micrograms per milliliter relative to a reference curve of cat serum (2 μ g of albumin per milliliter).

Depletion of cat-dander extract of *Fel d I*

By mouse monoclonal antibodies. One milliliter of monoclonal anti-*Fel d I* ascites (Fd-1a) was coupled to 300 mg of CNBr-activated Sepharose. Five hundred microliters of cat-dander extract (ARTU) was incubated overnight with the monoclonal anti-*Fel d I* Sepharose in a final volume of 4.5 ml; this supernatant is referred to in the tables as "Sup (monoclonal depletion)." The depletion was performed twice; 2 ml of the supernatant was again incubated overnight with the monoclonal antibody Sepharose. As a control, an equal amount of cat-dander extract (ARTU) was incubated twice with 300 mg activated-inactivated Sepharose. The supernatant of this mixture is called "sham-absorbed extract."

By polyclonal rabbit antibodies. Rabbit antiserum was produced by immunization with *Fel d I*, affinity purified as described above with the monoclonal antibodies. The specificity of this antiserum was tested by immunoblotting and in a double diffusion technique. Twenty milliliters of polyclonal rabbit antiserum R46 was incubated overnight with 1 ml normal cat serum. This incubation was performed to remove trace amounts of contaminating antibodies to cat serum albumin. Next, cat Sepharose was prepared by coupling proteins from 20 ml of 10% extract of cat epithelium to 2 gm of CNBr-activated Sepharose. The absorbed antiserum was incubated with cat Sepharose for 2 hours. After centrifugation and washing with PBS, the Sepharose-antibody complex was layered on a G25 (Sephadex) column (75 ml). Next, the column was washed with 150 ml PBS

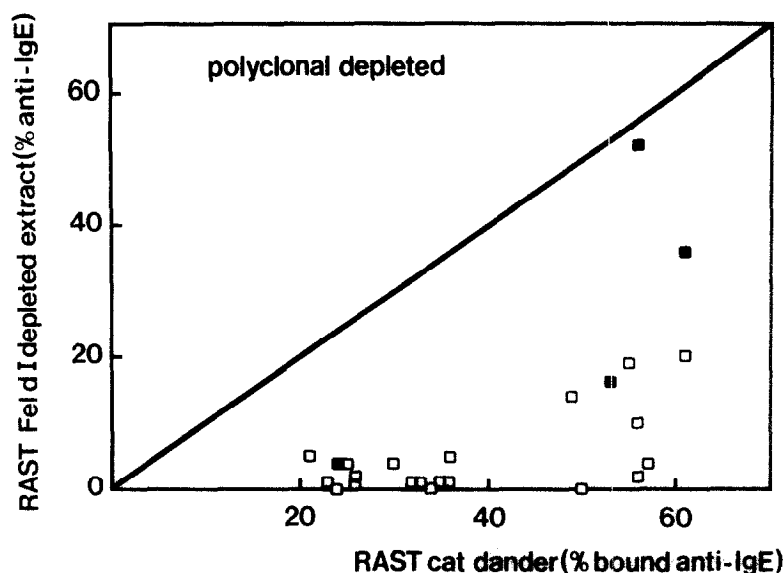


FIG. 3. Comparison between cat-dander RAST and *Fel d I*-depleted RAST; the *Fel d I*-depleted extract was made by a single absorption with polyclonal rabbit antibodies. Results are illustrated as percentage bound radioactivity (^{125}I -labeled anti-IgE). The sera from four patients with a high IgE response to cat serum are indicated by ■.

and elution was carried out with 20 ml of 0.065 mol/L glycine HCl (pH 2.5); this procedure yielded 2.6 mg protein. Rabbit anti-*Fel d I* (1.7 mg) was coupled to 100 mg of CNBr-activated Sepharose. Six hundred milligrams of this Sepharose was mixed overnight with 2.8 ml of cat-dander extract (200 FDA units *Fel d I*). This supernatant solution is referred to as "Sup (polyclonal depletion)."

The depleted extracts were coupled to CNBr-activated Sepharose for the RASTs. The concentrations used for coupling were chosen such that the amount of cat albumin in the different Sepharoses equaled the concentration of cat albumin in the cat-dander Sepharose.

Cat RAST

RASTs were performed with 250 μl of allergen Sepharose in a concentration of 2 mg Sepharose per milliliter and 50 μl of serum. RAST with cat dander (ARTU) was used as the reference test, as ARTU cat dander was the source of the *Fel d I* purification and depletion experiments. RAST occurred with purified *Fel d I* extract and with depleted extract to determine the contribution of *Fel d I* allergen to the IgE response with whole cat-dander extract. These extracts were obtained, as indicated above, from absorption with either rabbit polyclonal antibodies or mouse monoclonal antibodies.

For coupling to 100 mg activated Sepharose, we used 0.05 ml cat-dander extract (i.e., 3.5 U *Fel d I* and 6 μg cat albumin). To correct for dilution effects in the case of *Fel d I*-depleted extracts, the volume of the extract added was calculated on the basis of the cat albumin content: 6 μg cat albumin per 100 mg activated Sepharose, which corresponds to 0.18 and <0.02 U *Fel d I* for the monoclonal- and polyclonal-depleted extracts, respectively. Of the

affinity-purified *Fel d I*, 3.5 U was added, which contained <0.2 μg cat albumin. Coupling efficiency was $>90\%$.

Histamine release test

The histamine release test was performed according to Lichtenstein and Osler.¹⁸ Histamine content in the incubated samples was measured with an automated fluorometric method.^{19, 20}

RESULTS

Specificity of monoclonal antibodies for *Fel d I*

Results of the experiments with the CIE technique are shown in Fig. 1. The electrophoretic mobility of *Fel d I* is markedly reduced in the gels containing Fd-1a or Fd-1b compared with the gel containing control ascites Dp-1 (an anti-*D. pteronyssinus* antibody). It is concluded that both monoclonal antibodies Fd-1a and Fd-1b react equally with *Fel d I*. Fd-3 reacts with Ag3, i.e., cat serum albumin.

Furthermore, one can detect a "new" arc below the gel intersection that presumably represents an unretarded fraction of *Fel d I* (Fig. 1, A and B).

Based on crossinhibition experiments, we concluded that the two monoclonal antibodies were directed against different epitopes: significant cross-inhibition was found, but autoinhibition was much stronger than cross-inhibition. Moreover, in a two-site assay with radiolabeled Fd-1a, a good response was obtained when we used Fd-1b as capturing antibody

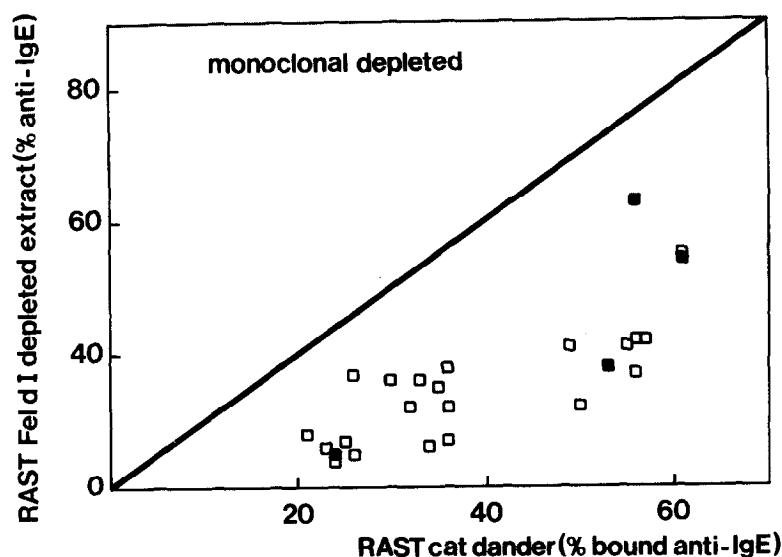


FIG. 4. Comparison between cat-dander RAST and *Fel d I*-depleted RAST; the *Fel d I*-depleted extract was absorbed twice with monoclonal antibodies. Results are illustrated as percentage-bound radioactivity (^{125}I -labeled anti-IgE). The sera of four patients with a high IgE response to cat serum are indicated by ■.

Fd-1b, whereas Fd-1a was ineffective. In contrast, Fd-1a was the more effective capturing antibody with radiolabeled *Fel d I*. In a double-antibody assay, the affinity constants for Fd-1a, Fd-1b, and polyclonal antibodies (ALK) were found to be 0.7, 0.04, and 0.6 L/U, respectively; assuming a conversion factor of 4 μg *Fel d I*/U and a molecular mass of 39 kd,²¹ these values correspond with 7, 0.4, and 6×10^9 L/mol.

Immunization of rabbits with affinity-purified *Fel d I*

Rabbits were immunized with monoclonal-purified *Fel d I* and the antiserum was tested in the double-diffusion technique. Only one precipitin line appeared, which fused with the line produced by the monospecific anti-*Fel d I* (Fig. 2).

Cat RAST

The amounts of *Fel d I* and cat albumin in the supernatants obtained after the depletion experiments were determined (Table I). The RAST with whole cat dander was almost identical to the RAST with the purified *Fel d I* extract and with the sham-absorbed cat-dander extract. The mean ratio (anti-IgE bound to cat dander) versus (anti-IgE bound to purified *Fel d I*) was 0.97 (range 0.80 to 1.14).

After *Fel d I* depletion by polyclonal rabbit antibodies, a marked decrease was found in all but two sera samples (Fig. 3). Sera that were less affected by

the depletion were found to have a high IgE-antibody titer to cat serum.

Similar but less striking results were found for monoclonal antibody depletion (Fig. 4).

Histamine release test

The following extracts were used to determine the relative sensitivity of the basophil leukocytes of patients allergic to cats to the purified major cat allergen: whole cat dander, purified *Fel d I* (dilutions were chosen to contain concentrations of *Fel d I* equal to the cat dander), and *Fel d I*-depleted extract, derived from the polyclonal and monoclonal antibody absorption (dilutions were chosen to contain equal concentrations of cat albumin).

The results of the histamine release test with the leukocytes of two patients allergic to cats are shown in Fig. 5. The histamine release from washed leukocytes, induced by purified *Fel d I*, is as strong as that induced by whole cat dander. *Fel d I* depletion results in a marked reduction in histamine release. The concentration required for the same histamine release compared with the whole cat-dander extract was 300 times (Fig. 5, A) or 200 times (Fig. 5, B), respectively, higher with the polyclonal depletion (*Fel d I* depletion factor of 330). The reduction factors are 40 and 30, respectively, with the monoclonally depleted extracts (*Fel d I* depletion factor of 20).

The third patient we tested with the histamine release test gave no interpretable data, due to a very

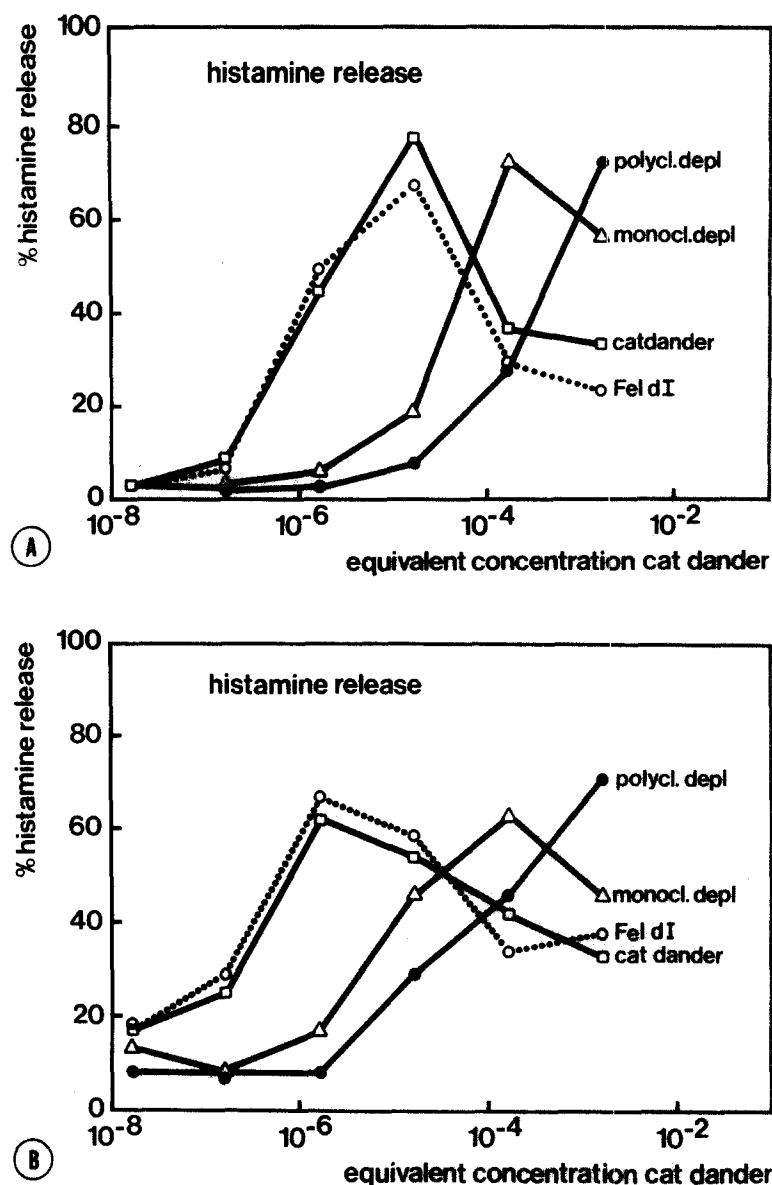


FIG. 5. Histamine release from basophil leukocytes in two patients with cat allergy (A and B). The concentration of cat dander is expressed as the dilution of a 10% cat-dander extract (obtained from ARTU). The concentration of the purified *Fel d I* was adjusted on the basis of the *Fel d I* content of the cat-dander extract. The concentrations of the depleted extracts were adjusted on the basis of the cat-albumin content. Results are illustrated as percentage histamine released.

high spontaneous release (65%). The fourth patient was characterized by a low maximal release with cat dander; the effect of depletion in this patient was a factor of 10 for the polyclonal depletion and a factor of 5 for the monoclonal depletion.

DISCUSSION

Monoclonal antibodies

We were able to isolate two monoclonal antibodies directed to a major allergen, *Fel d I*. The specificity of the monoclonal antibodies for *Fel d I* was dem-

onstrated by the following tests: (1) in CIE experiments (Fig. 1); (2) by RASTs, in which we found a high specificity of the two monoclonal antibodies to cat dander and no crossreactivity with nonfeline products was demonstrable; (3) by SDS-PAGE of ^{125}I -labeled cat antigen, which showed that the two monoclonal antibodies were found to react with a major band at 18 kd.²¹ This agrees with the results obtained by Leiternann et al.,⁶ who found a relative molecular mass of *Fel d I* of approximately 35 kd on a Sephadex G75 column but found a molecular weight of 18 kd

under the dissociative conditions of SDS-PAGE, suggesting that the native allergen comprises two subunits; and (4) with rabbit antisera obtained by immunization with monoclonally affinity-purified antigen, which formed a single immunoprecipitate in the double-diffusion technique; this line fused with the precipitin line formed by the anti-*Fel d I* antibody, provided by Dr. H. Baer.

The effect of *Fel d I* depletion

Studies performed by Ohman et al.^{22, 23} already indicated a major role of *Fel d I*. They found >90% decrease in skin test reactivity in patients allergic to cat after the absorption of the cat-dander extract with a polyclonal antiserum.

We performed several assays with *Fel d I* affinity purified by polyclonal antibodies and with cat extracts that were depleted of *Fel d I*. The amount of *Fel d I* left after depletion was 330 or 20 times less for the polyclonal and monoclonal depletion, respectively, compared with the whole cat-dander extract before depletion. The purified *Fel d I* and the *Fel d I*-depleted extracts were coupled to a solid phase to evaluate the specific IgE response from 25 patients with cat allergy to the different extracts. When depletion was performed with the polyclonal rabbit antibodies, we found a reduction in the cat-specific IgE response of 76% (range 7% to 100%); the sera samples with no reduction in the RAST score were characterized by a high IgE response to cat serum. When samples were depleted with monoclonal mouse antibodies, we found a less pronounced but still very marked decrease in IgE binding (average, 59%; range, 23% to 83%). Even after a second depletion step, residual *Fel d I* was still demonstrable. The importance of *Fel d I*, clearly demonstrated by the RAST on the cat panel, was furthermore confirmed with a biologic assay.

For this purpose, we performed a histamine release test on leukocytes from four patients allergic to cats. The basophil leukocytes were incubated with cat dander, purified *Fel d I* extract, and the depleted *Fel d I* extracts and histamine release was measured. No significant difference was found between the release after incubation with whole cat-dander extract and the release after incubation with the purified *Fel d I* extract, suggesting that the major allergen *Fel d I* indeed plays a major role in the allergic reaction to cat. As in the RAST, we measured a decrease in allergenic potency by a factor of 300, or a factor of 200 after depletion with polyclonal rabbit antibodies. With the monoclonal mouse antibody-depleted material, we measured a less pronounced but still highly significant reduction of the allergenicity (factors of 40 and 30,

respectively; more is necessary for the same histamine release). These results are very close indeed to the reduction factor of the *Fel d I* content in the depleted extracts.

Considering the results obtained with the histamine release test, we also found striking evidence for the fact that *Fel d I* is the major allergen of cat extracts. These results with cat dander are in marked contrast to the results obtained in the *D. pteronyssinus* system. Upon depletion of house-dust-mite extracts with antibodies to *Der p I*, van der Zee et al.¹⁰ did not find a considerable reduction in allergenic activity, either with monoclonal or with polyclonal antibodies. The effect of *Der p I* depletion was studied in the histamine release and the skin tests. Although RAST results showed a major role of *Der p I*, the histamine release test showed no differences between the release after incubation with the whole-mite extract or that after incubation with the *Der p I*-depleted extracts.

Difference between polyclonal and monoclonal depletion

It proved to be unexpectedly more difficult to achieve antigen depletion with the monoclonal antibodies than with the polyclonal antibodies. When the depletion was monitored with a monoclonal antibody-based assay, the apparent *Fel d I* level in the monoclonally depleted extract was lower than in the routinely used competitive radioimmunoassay, in which polyclonal rabbit antibodies were used. This made us wonder whether the polyclonal test might perhaps be less specific than we assumed. Therefore, we repeated some of the assays with radiolabeled *Fel d I* that was further purified by a second affinity step, subsequent to the iodination. This labeled *Fel d I* was >90% reactive with Fd-1a. Even with this labeled preparation, the dose-response curve of the monoclonally depleted extract in the polyclonal inhibition assay was indistinguishable from the reference *Fel d I*, indicating that the inhibitory material really was *Fel d I*. Support for this conclusion was obtained with Sepharose-coupled, affinity-purified *Fel d I* with human IgE antibodies in a RAST-inhibition assay. Again, the dose-response curve of the monoclonally depleted extract was indistinguishable from that of the reference *Fel d I*, and the calculated *Fel d I* level was, as expected, due to the polyclonal inhibition assay.

These data indicate that the monoclonally depleted extract contains some *Fel d I* (approximately 5%) that does not react with Fd-1a antibodies. One possibility is that this is due to the presence of antigenically deficient *Fel d I*, i.e., *Fel d I* that lacks a small part to which the monoclonal antibodies are directed, pos-

sibly a breakdown product. The presence of this variant of *Fel d I* (with different binding to monoclonal antibodies) was also observed in the CIE experiments.

Another possibility is that this effect is due to "bleeding" of the monoclonal antibodies off the affinity matrix. The depleted extract was indeed found to contain some mouse IgG, possibly monoclonal antibodies complexed with antigen. The *Fel d I* in these complexes would not be detected in the monoclonal antigen assay, but will be detected in both IgE-based assays and in the polyclonal assay, because these assays were found not to be inhibitable by the monoclonal antibodies.

We conclude from this study that in the cat dander system there is a major contribution of a single allergen, *Fel d I*. Therefore, the cat dander system may serve as a good model for the study of the human immune response to an inhalant allergen.

This study also illustrates the usefulness and limitations of applying monoclonal antibodies to study the effects of allergen depletion. In the cat dander system, monoclonal antibodies were certainly effective for allergen depletion (>90% depletion was easily achieved), but the depletion needs to be monitored carefully.

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REFERENCES

1. Aalberse RC. Allergens in house dust. *Acta Otorhinolaryngol Belg* 1978;32:25.
2. Bryant DH, Burns MW. Skin test reactions to inhalant allergens in asthmatic patients. *Med J Aust* 1976;1:918.
3. Sarsfield JK, Boyle AG, Rowell EM, Moriarty SC. Pet sensitivities in asthmatic children. *Arch Dis Child* 1976;51:186.
4. Kurata JH, Glovsky MM, Newcomb RL, Easton JG. A multifactorial study of patients with asthma. II. Air pollution, animal dander and asthma symptoms. *Ann Allergy* 1976;37:398.
5. Schwartz B, Lind P. Immunochemical method for investigations of allergic patients' environment. Results of elimination treatment of the allergen source. *J ALLERGY CLIN IMMUNOL* 1984;74(suppl):156.
6. Leitermann K, Ohman JL. Cat allergen. I. biochemical, antigenic and allergenic properties. *J ALLERGY CLIN IMMUNOL* 1984;74:147.
7. Lowenstein H. Allergen von Katze. *Hund, Rind und Pferd. Allergol Jahrgang* 1981;4:265.
8. Lowenstein H, Lind P, Weeke B. Identification and clinical significance of allergenic molecules of cat origin. *Allergy* 1985;40:430.
9. Lowenstein H. Quantitative immunoelectrophoresis methods as a tool for the analysis and isolation of allergens. *Prog Allergy* 1978;25:62.
10. van der Zee JS, van Swieten P, Jansen HM, Aalberse RC. Skin tests and histamine release with P₁-depleted *D. pteronyssinus* body extracts and purified P₁. *J ALLERGY CLIN IMMUNOL* 1988;81:884-96.
11. Hunter WM, Greenwood FC. Preparation of iodine-131-labeled growth hormone of high specific activity. *Nature* 1962;194:495.
12. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975;256:495.
13. Kohler G, Milstein C. Derivation of specific antibody-producing tissue culture and tumour lines by cell fusion. *Eur J Immunol* 1976;6:511.
14. Astaldi GCB, Jansen MC, Lansdorp P, Willems C, Zeijlemaker WP, Oosterhof F. Human endothelial culture supernatant (HECS): a growth factor for hybridoma. *J Immunol* 1980;125:1411.
15. Aalberse RC. Monoclonal antibodies in allergen standardization. In: *Arbeiten aus dem Paul-Ehrlich-Institut, dem Georg-Speyer-Haus und dem Ferdinand-Blum-Institut*. Heft 7B. Stuttgart/New York: Gustav Fischer Verlag, 1983:137.
16. Lind P. Purification and partial characterization of two major allergens from the house dust mite *Dermatophagoides pteronyssinus*. *J ALLERGY CLIN IMMUNOL* 1985;76:753.
17. Sutherland Ch. The preparation of house dust extracts. *Br J Med* 1942;2:280.
18. Lichtenstein LM, Osler AG. Histamine release from human leucocytes by ragweed-pollen antigen. *J Exp Med* 1964;120:507.
19. Siraganian RP. An automated continuous flow system for the extraction and fluorometric analysis of histamine. *Anal Biochem* 1974;57:383.
20. Siraganian RP. Refinements in the automated fluorometric histamine analysis system. *J Immunol Methods* 1975;7:283.
21. 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.
22. Ohman JL, Lowell FC, Bloch KJ. Allergens of mammalian origin. III. Properties of a major feline allergen. *J Immunol* 1974;113:1668.
23. Ohman JL, Lowell FC, Bloch KJ, Kendall S. Allergens of mammalian origin. V. Properties of extracts derived from the domestic cat. *Clin Allergy* 1976;6:419.