Identification, characterization, and cloning of a complementary DNA encoding a 60-kd house dust mite allergen (Der f 18) for human beings and dogs

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Background: House dust mites of the *Dermatophagoides* genus are the most important cause of perennial allergic disease in both humans and companion animals. Although the major mite allergens for humans are proteins of relatively low molecular weight, this is not the case for dogs. Western blotting shows that canine anti-mite IgE responses are directed primarily toward proteins in the molecular weight range of 50 to 120 kd.

Objective: The objectives of this study were to characterize a *D farinae* allergen with a molecular weight of approximately 60 kd and to isolate the cDNA coding for this allergen.

Methods: A protein of apparent molecular weight of 60 kd was identified by Western blotting by using canine serum IgE from house dust mite–sensitized atopic dogs. The protein was purified from homogenized *D farinae* mite bodies by ammonium sulfate precipitation, followed by gel filtration and cation exchange HPLC. The presence of IgE directed to the 60-kd protein in sera from humans and dogs with dust mite allergy was measured by FcεRIα-based ELISA. A cDNA encoding a full-length 60-kd protein was isolated from a *D farinae* cDNA library by a combination of both PCR amplification and hybridization screening. A panel of mAbs specific for the 60-kd protein was generated and used to localize the protein in whole body sections of *D farinae* mites.

Results: ELISA showed that the purified protein bound IgE in 54% of the sera from patients with *D farinae* allergy. In addition, the 60-kd protein was able to bind IgE in 57% to 77% of *D farinae*–sensitized dogs. A cDNA was isolated that encoded a protein of 462 amino acids, consisting of a 25 amino acid signal sequence and a 437 amino acid mature protein. The calculated molecular weight of the mature protein is 50 kd, and the amino acid sequence contains a single N-glycosylation site. A protein database search showed homology with multiple chitinases. A mAb specific for the 60-kd chitinase recognized the allergen in the mite digestive system, but fecal pellets did not stain positively for this allergen.

Conclusions: A 60-kd *D farinae* protein (Der f 18), with homology to chitinase, is a major allergen for humans and dogs sensitive to house dust mites. (J Allergy Clin Immunol 2003;112:79-86.)

Key words: *House dust mite, allergen, ELISA, cDNA cloning, monoclonal antibodies, immunomapping*

House dust mites are the most important indoor allergens for humans. House dust mites are also important allergens for dogs with atopic dermatitis, and high rates of positive intradermal test results to mite extracts have been reported in dogs.[1] Atopic dermatitis in the dog has many similarities to that in humans, including an inherited predisposition, early age of onset, similar distribution of affected skin, and the tendency to mount IgE responses to common allergens. The dog is thus a good model for atopic dermatitis in humans.

The best characterized mites known to elicit IgE responses in both humans and dogs are *Dermatophagoides pteronyssinus* and *D farinae*. Human IgE antibodies to the mite allergens are significantly cross-reactive, and most individuals react to both *D farinae* and *D pteronyssinus*. In contrast to humans, dogs show higher rates of positive skin test results to *D farinae*, even in regions in which both species are endemic.[2,3]

Major mite allergens for humans have been the subject of intensive research. Western blotting studies with human sera containing high levels of anti-mite IgE showed 32 bands with molecular weights ranging from 11 to greater than 100 kd.[4,5] To date, at least 15 *D farinae* allergens have been characterized and their cDNAs cloned, with the great majority of these being in the molecular weight range of 14 to 60 kd.[6,7] The most extensively characterized mite allergens are the group 1 and group 2 molecules, which are a 25-kd cysteine protease[8] and a 14-kd epididymal protein,[9] respectively. More than 80% of humans with house dust mite allergy mount an IgE response to the group 1 molecules and more than 90% to the group 2 allergens. In contrast, these molecules do not appear to be the major allergens for dogs, as measured by Western blotting, in vitro IgE assays, or intradermal skin testing.[10-12] Canine anti-mite IgE

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Abbreviation used
FcεRIα: Fc epsilon receptor 1 alpha chain

appears to be directed mainly against mite extract components of higher molec-ular weight. Therefore, the allergen profile of the IgE response of dogs to house dust mites is different from that of humans. Previous studies have shown that a high percentage of atopic dogs have IgE specific for a *D farinae* chitinase (Der f 15) of apparent molecular weight of 98 kd.[7] More recently, Western blotting studies have shown that a high percentage of dogs with *D farinae* allergy also have IgE directed at a protein with a molecular weight of approximately 60 kd. The aim of this study was to purify this 60-kd protein, to characterize its role as an allergen for both humans and dogs, and to isolate the cDNA encoding this allergen.

METHODS

Plasma and serum samples

Human plasma samples were purchased from PlasmaLab International (Everett, Wash). Samples were characterized as being either negative or positive for IgE against *D farinae* by RAST.

Canine sera were obtained from 2 groups of animals. The first group consisted of pet dogs with atopic dermatitis that were admitted into the Dermatology Service, College of Veterinary Medicine, at The Ohio State University. As part of their clinical evaluation, these animals were intradermally tested under sedation (xylazine hydrochloride and atropine sulphate) by using standard procedures,[13] with both crude *D farinae* extract (Greer Laboratories, Lenoir, NC) (1:50,000 w/v concentration) and purified 60 kd allergen (1 μg). Immediate skin test reactivity (erythema and wheal size) was assessed after 15 minutes and scored on a scale of 0 to 4, where 0 was equivalent to sterile diluent negative control and 4 was equivalent to histamine positive control. A score of 2 or greater was regarded as positive. The second group was composed of a cohort of pet dogs, seen by general practice veterinarians, with a putative diagnosis of atopic dermatitis. Sera from these animals were submitted to Heska's Veterinary Diagnostic Laboratories for IgE testing. Intradermal test data were not available for these animals.

ELISA

An ELISA with a recombinant form of the extracellular domain of the human high affinity IgE receptor alpha chain (rFcεRIα) as IgE detection reagent was carried out as previously described.[14] Briefly, whole mite extract (Center Labs, Port Washington, NY) or purified native Der f 18 was coated onto 96-well plates in carbonate/bicarbonate buffer and incubated overnight at 4°C. The plates were then blocked with assay buffer (PBS + 4% v/v fetal bovine serum + 0.02% v/v Tween-20) for 30 minutes at room temperature. After removal of the blocking solution, 100 μL of a 1:20 v/v dilution of serum or plasma in assay buffer was added to duplicate wells, incubated overnight at 4°C, and washed 4 times on an automated plate washer. After washing, an optimized dose of biotinylated recombinant FcεRIα was added for 2 hours at room temperature. The plates were washed, and 10 ng per well streptavidin-conjugated horseradish peroxidase (KPL Labs, Gaithersburg, Md) in 100 μL assay buffer was added for 1 hour at room temperature. The plates were developed with 100 μL per well 3,3´,5,5´ tetramethylbenzidine substrate for 30 minutes at room temperature, and the optical density was measured at 450 nm. Optical density from background wells (no serum) was subtracted, and the results were expressed as (Test optical density – Background optical density) × 1000. Corrected optical densities of 100 or greater were considered to be positive (cutoff defined as mean plus 3 standard deviations of values of multiple heat inactivated nonallergic samples).

Purification of Der f 18

Ten grams of whole *D farinae* bodies was washed, suspended in 50 mmol/L NaOAc buffer (pH 4) containing 0.15 mol/L NaCl, and homogenized in a Polytron homogenizer (Brinkman, NJ). The suspension was centrifuged at 29,000*g* for 15 minutes, and the supernatant was subjected to ammonium sulfate precipitation. The 40% to 60% (w/v) ammonium sulfate precipitate was collected and dissolved in 10 mL of the same buffer and subjected to gel filtration chromatography on an S-100 (2.5 × 70 cm) sizing column pre-equilibrated in the same buffer. Fractions (5 mL) were collected and analyzed by SDS-PAGE gels (14% w/v), and fractions containing a 60-kd protein were concentrated and fractionated on an ion-exchange Q-Sepharose column (1 mL). The column was eluted with a step gradient (0.020 to 1 mol/L) of Tris-HCl (pH 8). Fractions containing a 60-kd protein, as determined by SDS-PAGE, were collected, and the pH was adjusted to 5.5 with acetic acid and conductivity adjusted to 3 to 4 mυ with water. The sample was applied to a PolyCat cation exchange HPLC Column (PolyLC, Columbia, Md) pre-equilibrated in 0.020 mol/L NaOAc (pH 5.5) and eluted with a gradient of NaCl in the same buffer. After elution, fractions were analyzed for the presence of Der f 18 by SDS-PAGE. Molecular size markers (Mark 12 unstained standards; InVitrogen, Carlsbad, Calif) were used. Western blotting was performed on proteins transblotted to polyvinylidene difluoride (PUDF) membrane by using pooled positive sera followed by a biotinylated anti-canine IgE mAb (H207), streptavidin–horseradish peroxidase and ECL chemiluminescent detector (Amersham Biosciences, Piscataway, NJ).

Amino terminal and internal peptide sequence analysis

The purified 60-kd protein was blotted onto polyvinylidene difluoride membrane and stained with Coomassie R-250 by using standard procedures. A protein band of approximately 60 kd was excised and subjected to N-terminal amino acid sequencing, as previously described.[7] The protein corresponding to the 60-kd region was also subjected to proteolytic cleavage to obtain internal amino acid sequence data. Digestion of the 60-kd protein and reverse-phase chromatography were carried out as previously described.

cDNA cloning

Five micrograms of *D farinae* poly A+ mRNA was used to construct a cDNA library in lambda Uni-Zap XR vector (Stratagene, La Jolla, Calif) by using Stratagene's Zap cDNA Synthesis Kit protocol. A degenerate, oligonucleotide primer (5´-GAACCAAAA CHGTNTGYTA YTAYG-3´, Y=C/T, H=A/C/T) corresponding to amino acid residues 3 through 11, based on the amino terminal sequence of Der f 18, was used in conjunction with the M13 forward universal primer in a primary PCR reaction. The product of this PCR reaction was nested with the T7 standard primer and a second primer (5´-GATATGGAACATTTYACHCAACAYAARGG-3´, Y=C/T, H=A/C/T, R=A/G) corresponding to a region spanning from amino residue 1 through amino acid residue 10 of Der f 18 internal amino acid sequence. A 510-bp PCR product was generated and was shown by DNA sequence analysis to encode a portion of Der f 18. With a modification of the plaque hybridization protocols,[15] the *D farinae* cDNA library was screened by using duplicate plaque lifts, with a ^{32}P-labeled cDNA encoding the 510-bp PCR product encoding a portion of Der f 18. Approximately 1.0 × 10^6

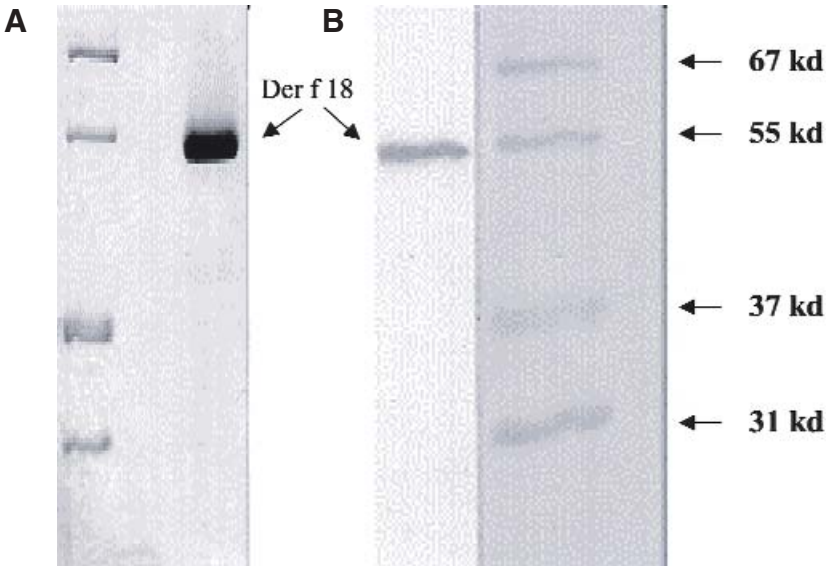

FIG 1. A, Coomassie-stained PAGE of purified Der f 18. Two micrograms of purified Der f 18 was resolved on a 14% SDS reducing PAGE gel, stained with Coomassie Blue, and destained. **B,** Canine IgE Western blot of purified native Der f 18. Approximately 5 ng of purified Der f 18 was resolved on a 14% SDS reducing PAGE gel.

plaques were screened; positive plaques were picked and purified by further rounds of screening; and phagemid DNA was isolated according to the manufacturer's instructions (Stratagene, La Jolla, Calif). Plaque-purified clones of the *D farinae* nucleic acid molecules encoding Der f 18 were converted into double-stranded recombinant molecules and subjected to DNA sequence analysis by dideoxy sequencing. Each strand of the cDNA was sequenced twice to generate nonambiguous sequence.[16]

DNA sequence analysis

DNA sequence analysis, including the alignment of sequences and the determination of open reading frames, was performed by using the MacVector program (IBI, New Haven, Conn). Protein sequence analysis, including the determination of molecular weight and isoelectric point, was performed by using the GCG program (Genetics Computer Group, Madison, Wis).

Preparation of mAbs against Der f 18 and immunomapping of the protein in whole body sections of *D farinae* mites

One of a panel of 6 mAbs generated against the purified Der f 18 (H407, IgG1 isotype) was used for immunostaining. Briefly, paraffin-embedded sections of whole mite cultures were deparaffinized, hydrated, and then incubated with 0.25% w/v pepsin (Sigma Chemicals, St Louis, Mo) for 30 minutes at room temperature for antigen retrieval. The sections were rinsed in PBS, overlaid with 3% v/v hydrogen peroxide for 5 minutes, and rinsed in PBS. Blocking of nonspecific immunoreactivity was carried out with a solution containing 1% v/v newborn calf serum and 1% skim milk for 20 minutes, followed by rinsing in PBS. Sections were then incubated with H407 (1:100 dilution for 1 hour at room temperature), rinsed in PBS, and then incubated with secondary antibody (biotinylated horse anti-mouse IgG [Vector Lab, Burlingame, Calif]) in the presence of streptavidin peroxidase (Zymed, South San Francisco, Calif). Aminoethylcarbazole was used as red chromogen. The negative control was an IgG1 mAb specific for canine CD8.

RESULTS

Purification and internal sequence analysis of the 60-kd allergen

A purified preparation of Der f 18 was obtained by using sequential fractionation on Sephacryl-S-100, Q-Sepharose, and PolyCat anion exchange HPLC (Fig 1). The purified 60-kd protein was digested with endoproteinase Asp-N, and the resulting peptides were separated on a C18 reversed phase HPLC column. To obtain N-terminal sequence of this protein, a portion of the purified material was resolved on a 14% SDS-PAGE gel and transferred to polyvinylidene difluoride membrane. The appropriate bands containing Der f 18 were cut out and submitted for N-terminal sequence analysis. The peptide sequences obtained, as well as the homologues identified in an NCBI Basic Local Alignment Search Tool Program (BLASTP) database search, are shown in Table I. The result of this search showed that peptides A15, 26a, 24a, 23b, and 26b have homology to regions within other chitinase or chitinase precursor molecules.

Frequency of Der f 18–specific IgE in atopic canine and human patients

Analysis of human sera. Twenty-nine plasma samples from atopic human patients with historically positive RAST scores to *D farinae* were purchased from a commercial supplier (PlasmaLab International, Everett, Wash). A negative plasma pool was prepared from samples obtained from healthy donors resident in Colorado (a house dust mite nonendemic area) that were negative to *D farinae* extract by ELISA. Table II shows data using the FcεR1α-based ELISA to measure IgE against mite

TABLE I. Der f 18p internal peptide sequences and associated Basic Local Alignment Search Tool Program results

Peptide number	Peptide sequence	Homologous protein	Region of homology	GenBank accession no.
A15	MTLEPKTV**CYYES WVHWRQGEGK**M	Chitinase (*Acanthocheilonema viteae*)	22-36	AAC47022
26a	**DTSLCTHIVYSYFGIDA** ATH	Chitinase (*Drosophila melanogaster*)	49-66	AAL49181.1
15b	DMEHFTQHKGNAKA	No homology		
7	DQFSKTAAVEHYR	No homology		
5	DWSGMQAK	No homology		
24b	DNFIKL	No homology		
24a	VMGVTLPATIASYDN **YYNIPAISNYVDFMNVL**	Chitinase (*Haemaphysalis longicornis)*	219-234	Q8MY79
23b	**DYTGSWAHTVG**HASPFPEQ	Chitinase (*Aspergillus nidulans*)		
3	DKASGPGPR	No homology		
26b	**DGFLSYNELC**VQIQAETN	Chitinase precursor (*D melangaster*)	312-321	Q9W092
16b	DVHGVCGDKNPLLHAIQSN YYHGVVTEPTVTLPPVTH	No homology		

Bolded sequences denote region of similarity with homologue.

TABLE II. Anti–*D farinae* extract IgE and anti–Der f 18 IgE in human plasma samples, measured by FcεRIα-based ELISA

Patient no.	*D farinae* extract	Der f 18
1	19	0
2	**2168**	**444**
3	**2170**	**470**
4	**1380**	5
5	79	74
6	**470**	1
7	**484**	48
8	**716**	**187**
9	**1309**	**144**
10	**1641**	**291**
11	**1365**	4
12	**411**	**228**
13	36	9
14	93	0
15	**866**	**757**
16	**980**	**139**
17	**249**	48
18	34	12
19	**144**	13
20	**109**	7
21	**375**	**153**
22	**1193**	23
23	**1070**	**480**
24	**758**	**194**
25	**2067**	**324**
26	**180**	53
27	**430**	2
28	**977**	69
29	**2493**	**3058**
Negative pool	7	0

Positive values are in bold.

extract and purified Der f 18. It was determined that 13 of 24 (54%) of the *D farinae* extract positive plasma samples were also positive to Der f 18.

Analysis of canine sera. FcεRIα-based ELISA and intradermal test results for 30 dogs with atopic dermatitis, by using purified 60-kd protein and *D farinae* extract, are shown in Table III. Nineteen (63%) of the dogs tested were positive to *D farinae* extract by intradermal test. Within this positive intradermal test group, 15 (79%) were also positive to the extract in an ELISA. Eighty percent (12) of the dogs that were both ELISA- and intradermal test–positive to *D farinae* extract also had IgE directed to the 60-kd protein, as measured by ELISA. However, the proportion of dogs with a positive intradermal test result to *D farinae* extract that were also skin test positive to the 60-kd protein was lower, 44%. In addition, none of the 11 dogs that were negative to *D farinae* extract by intradermal test were positive to the 60-kd allergen by either intradermal test or ELISA, although 6 of these dogs had IgE against *D farinae* extract as measured by ELISA. Furthermore, if the 6 dogs that were intradermal test negative but tested positive to mite extract by ELISA were grouped with the 15 dogs that were both intradermal test and ELISA positive, the rate of Der f 18–positive dogs by ELISA was 57% (12 of 21). The second group of canine sera tested were 35 samples submitted to a commercial diagnostic laboratory for a 48-allergen panel test and found to be positive for *D farinae*. Within this group 27 (77%) tested positive to Der f 18 by ELISA, with values ranging from 103 to 3275 (data not shown).

Cloning of cDNA encoding Der f 18

A cDNA encoding full-length Der f 18 (GenBank accession number AYO93656) was isolated from a *D farinae* cDNA library by a combination of both PCR amplification and hybridization screening. The cDNA encoding Der f 18 (Fig 2) is 1445 bp in length and contains an open reading frame of 1386 bp. The open reading frame encodes a protein of 462 amino acids, consisting of a putative 25 amino acid signal sequence[17] and a 437 amino acid mature protein. The calculated molecular

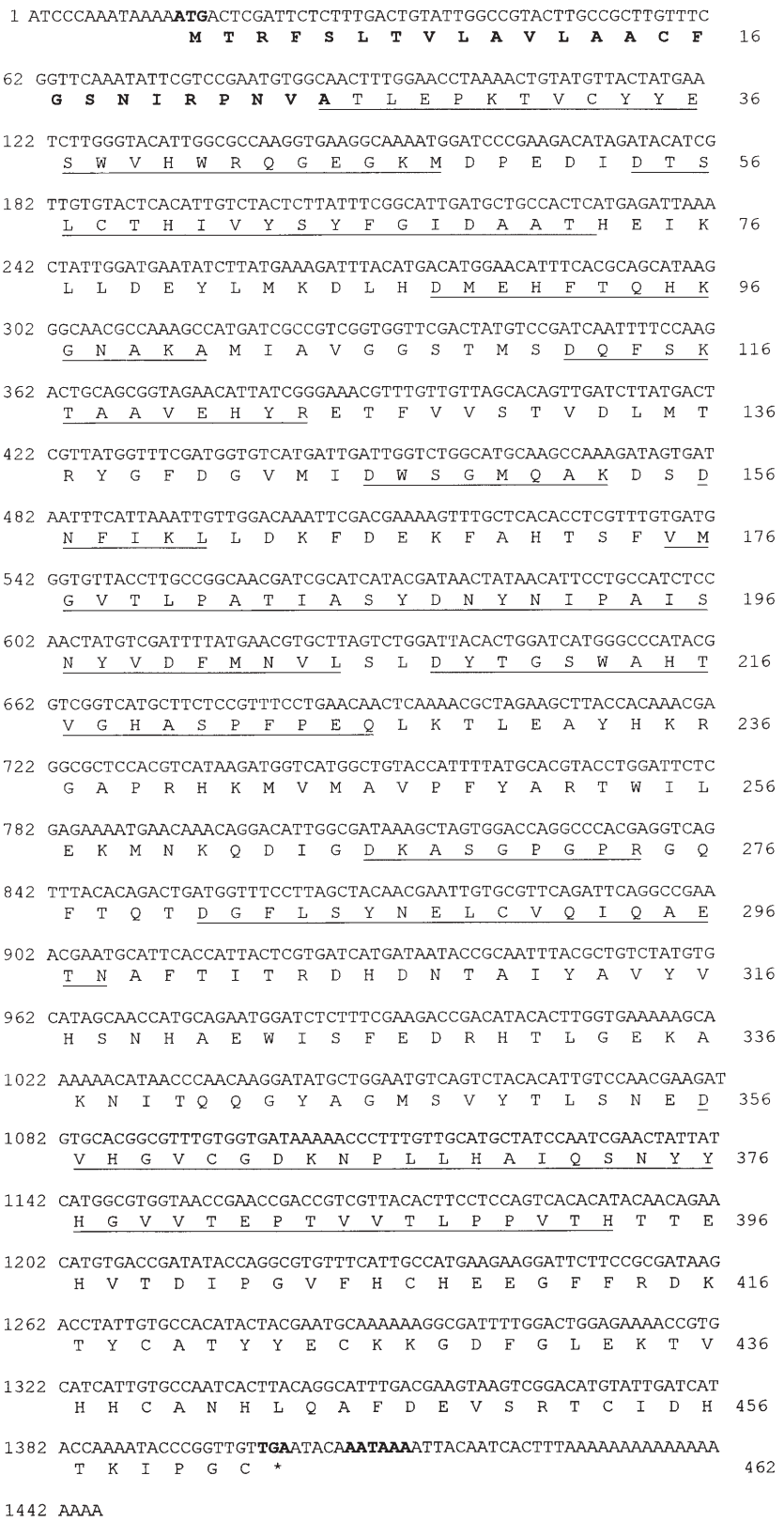

FIG 2. Nucleotide sequence of Der f 18 cDNA with deduced amino acid sequence. From *top to bottom*, the ATG initiation codon, amino acids encoding the secretory leader peptide, N-glycosylation site (N-I-T, aa 338-340), stop codon (TGA), and the putative polyadenylation signal (AATAAA) are in *bold*. Internal peptide sequences generated by protein sequencing are *underlined*.

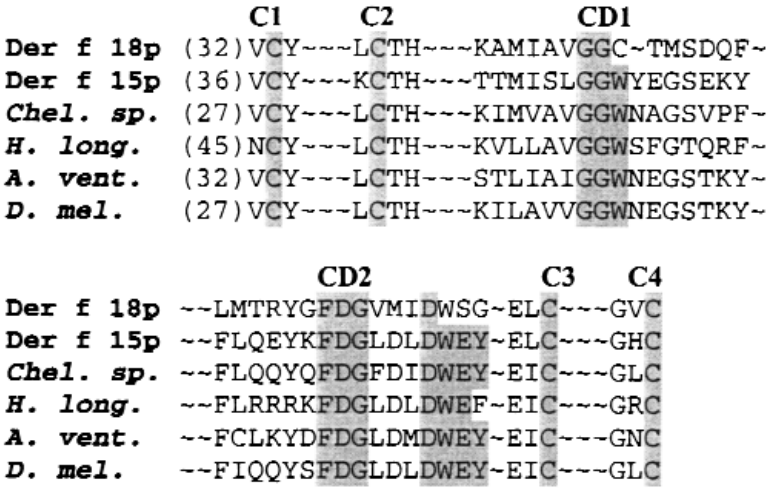

FIG 3. Regional protein sequence alignment of Der f 18 with homologues showing conserved cysteine residues, labeled C1 to C4. The conserved residues within the 2 catalytic domains are labeled CD1 and CD2, respectively.

TABLE III. Anti–*D farinae* extract IgE and anti–Der f 18 IgE in dogs, as measured by intradermal test and FcεRIα-based ELISA

	D farinae extract		Der f 18	
Dog	ELISA	Intradermal test	ELISA	Intradermal test
1	9	–	2	ND
2	89	–	0	–
3	**131**	–	33	–
4	**133**	–	75	–
5	64	–	17	–
6	**243**	–	75	–
7	**125**	–	44	–
8	8	–	0	–
9	**748**	–	92	–
10	80	–	22	–
11	**298**	–	39	–
12	**812**	+	**545**	ND
13	**4200**	+	**4200**	+
14	**213**	+	**139**	–
15	71	+	70	–
16	**254**	+	**107**	–
17	**222**	+	**118**	–
18	**293**	+	53	+
19	**150**	+	**147**	ND
20	74	+	**109**	ND
21	**894**	+	**121**	+
22	**145**	+	14	–
23	**1964**	+	**953**	+
24	15	+	7	–
25	**1692**	+	**401**	+
26	**1080**	+	**693**	–
27	43	+	48	–
28	**250**	+	**145**	+
29	**832**	+	**526**	+
30	**398**	+	40	–

+, Intradermal test score 2 or greater; –, intradermal test score less than 2; *ND*, not done. **Bolded** values considered positive.

weight of the mature protein is 50 kd, and the predicted isoelectric point is 5.61. In addition, the predicted protein contains a single putative N-glycosylation site. A search of the protein databases showed the protein to have 32% identity with a chitinase precursor from the wasp *Chelonus* species. In addition, this search showed several chitinase molecules with similar levels of identity from several species including *Drosophila melanogaster* and *Araneus venticosus*. Furthermore, an alignment of Der f 18p with Der f 15p shows 30% identity between the 2 proteins. Although these homologies are not extensive, there is conservation of cysteine residues as well as residues in the 2 catalytic domains (Fig 3).

Immunolocalization of Der f 18

Immunostaining of whole *D farinae* mite body sections performed with mAb staining showed that Der f 18 was present in the digestive system of the mites. In most cases, the staining was localized to the ventriculus (Fig 4), and in very rare acarids, it was also found in the intestine or esophagus. Remarkably, fecal pellets, either inside the rectum or defecated outside of the body, did not stain for this allergen.

DISCUSSION

In a growing percentage of the population of industrialized countries, house dust mites are becoming important indoor allergens, eliciting IgE antibody responses and allergic diseases such as rhinitis, asthma, and atopic dermatitis.[18] In addition, companion animals, which often share the environment of their owners, are also manifesting increasing rates of atopy, the major allergic disease affecting dogs. Intradermal testing with threshold doses of extract showed that approximately 70% of dogs suspected of atopy were sensitized to dust mites.[19] A

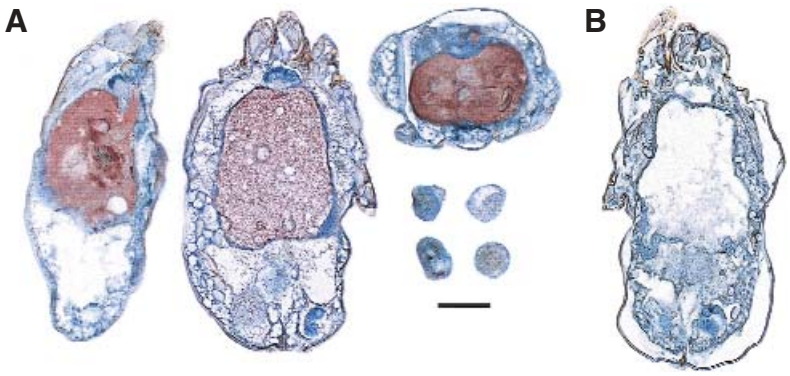

FIG 4. Immunostaining of Der f 18 in whole body sections of *D farinae*. **A,** Paraffin-embedded sections were immunostained with mAb H407 specific for Der f 18 by using a 3-step streptavidin-peroxidase method. This putative chitinase was localized to the upper digestive tract of the mites, such as the esophagus (as seen in the longitudinal section at *left*), or the ventriculus (as seen in the longitudinal, sagittal, and cross-sections of the mites). Immunostaining of the mites was carried out with an irrelevant mAb **(B)**. Of note is that in all longitudinal and sagittal mite sections, the cuticle covering the cranial body and proximal front exhibited a brown color. This color is considered normal for *D farinae* house dust mites. *Bar* = 60 μm.

similar incidence of sensitization to house dust mites was found in the population of putative atopic dogs whose sera were submitted for in vitro IgE testing to a commercial laboratory. Although house dust mites can elicit allergies in both humans and dogs, the profile of reactivity to mite allergens is very different between humans and dogs. Furthermore, in dogs *D farinae* appears to be more relevant than *D pteronyssinus*, even in areas in which both mites are present in the environment, indicating that the IgE responses mounted against the 2 mites often do not cross-react.[2,3]

Although less than 50% of dogs have IgE directed toward Der f 1 and Der f 2,[12] at least 80% of human subjects with allergy have anti–group I or group II allergen specific IgE.[6] Dogs appear to mount IgE responses to house dust mite allergens of higher molecular weight than humans. Western blot studies with canine IgE showed a profile of reactivity mainly to proteins of 60 kd and larger.[11,20,21] Previous studies in our laboratory have shown that one of these major allergens (Der f 15) is a putative chitinase with a molecular weight of approximately 98 kd.[7]

Western blot studies have shown that many canine sera contain IgE that binds to a protein of 60 kd apparent molecular weight. Serum IgE from approximately 50% of adult human patients also binds a 56- to 63-kd protein from *D pteronyssinus* (Der p 4), which has been shown to be an amylase.[22] However, in this study we have identified a unique 60-kd allergen recognized by canine IgE, which has homology to other arthropod and insect chitinases. In a group of well-characterized atopic dogs sensitized to *D farinae*, IgE to the purified 60-kd protein was found to be present in more than 50% of cases, as measured by ELISA. IgE to Der f 18 was detected more readily by ELISA than by intradermal testing in these animals. Dogs routinely have much higher total serum IgE concentrations than humans, up to 350 μg/mL.[23,24] Therefore, much higher concentrations of allergen are usually required to elicit skin mast cell degranulation than would be necessary in atopic humans. The rFcεRIα-based ELISA used in these studies measures allergen-specific IgE independently of total serum IgE concentration.[14] Therefore, depending on the physicochemical nature and epitope density of the allergen, specific IgE measurements made by in vivo or in vitro tests in dogs might not show concordance.

Serum IgE that binds Der f 18 is found in 13 of 24 (57%) of human subjects with anti–*D farinae* reactivity, as measured by ELISA. Therefore, it is a major allergen for humans as well as dogs and has been formally designated Der f 18 in the allergen nomenclature system. (The data characterizing the 60-kd chitinase have been submitted to the WHO/IUIS Allergen Nomenclature Subcommittee and the protein named Der f 18.)

Previous studies have shown that the high molecular weight allergen Der f 15 is homologous to chitinase. Analysis of the protein sequence of Der f 18 shows homology to several chitinase molecules from different organisms, making it the second mite allergen that is homologous to chitinase. Although the overall level of homology is not extensive, conservation of cysteine residues and residues in the 2 catalytic domains implies conservation of function. These data, in conjunction with the localization of both Der f 15 and Der f 18 to the mite gut, are suggestive that these proteins might play a role in digestion.[25]

Although Der f 18 has a predicted molecular weight of approximately 50 kd, it has a relative molecular mass of 60 kd. This discrepancy between the predicted and experimental molecular weight of the protein is not likely to be entirely due to glycosylation at the single N-glycosylation site on the protein, although deglycosylation of Der f 18 at N-linked sites does show a slight decrease in the experimental relative molecular mass (data not shown). In addition, deglycosylation of O-linked sugars on Der f 18 has no effect on the relative molecular mass. Therefore, it is likely that the observed difference between

experimental and predicted molecular weight of Der f 18 is due to other post-translational modifications or aberrant migration of the protein in the gel system used.

In contrast to the group I and II mite allergens that only elicit IgE responses in a minority of canine patients, Der f 18 is a major allergen for both humans and dogs. This disparity in allergen profiles might reflect differences in antibody or T-cell receptor repertoire, MHC molecules, or differences in the nature of antigen presentation. Epicutaneous allergen exposure might generate different responses than exposure via respiratory mucosa. Recent studies have shown that epicutaneous exposure is sufficient to induce anti–*D farinae* IgE in puppies, suggesting that the allergens important for the dog might not need to become airborne to effect sensitization.[26]

In conclusion, we describe here a new 60-kD allergen, from *D farinae*, that elicits detectable IgE in more than 50% of human and canine patients with house dust mite sensitivity tested. Further studies with a recombinant form of this allergen might enable us to better understand both the mechanism of sensitization by this allergen and the biologic role of this allergen in the house dust mite.

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