

Distribution of Core Fragments from the Major Cat Allergen Fel d 1 Is Maintained among the Main Anatomical Sites of Production

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Key Words

Anal sac • Fel d 1 • Allergen isoforms • Allergen extracts • Immunoreactivity • Structural polymorphism

Abstract

Background: Polymorphism of Fel d 1 has long been observed, but structural characterization of Fel d 1 variants among the various sites of production and animals is still missing. This study was aimed at elucidating the structural polymorphism of this protein as a function of the site of production and the resulting influence on the immunological behavior of the allergen. **Methods:** Fel d 1 was water-extracted from the 4 main sites of production, i.e. cheek zone, interdental zone, anal sac (AS), and chest area and a panel of 10 cats. Fel d 1-like materials were immunoblotted, immunopurified and characterized by MALDI-TOF MS. Immunoreactivity was studied using ELISA dose-dependent curves at equilibrium. **Results:** In all areas, 4–10 isoforms ranging from 7 to 40 kDa were detected by MS. Essentially two truncated heterodimers of 13–14 and 16–17 kDa were identified together with intact Fel d 1 in all compartments and they were largely predominant in AS. These core fragments were found to contribute to a variable recognition by antibodies in a panel of ELISA. **Conclusions:** Our study identified truncated

dimers of Fel d 1, probably resulting from proteolytic degradation of both chains and present in all cats. Core fragments are largely distributed among anatomical sites of production and especially well represented in AS. They are recognized as intact allergens by antibodies and may therefore introduce discrepancies in allergen measurement depending on the variable amount of intact and degraded Fel d 1 produced by the cat.

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Introduction

Allergy is a growing health problem and cat sensitization ranks first among pet allergies, with a prevalence of approximately 10% [1]. Fel d 1 is the major allergen of this animal. It is produced by lacrimal glands, salivary glands, sebaceous glands, secreted onto the skin and the fur of the animal, and present in large amount in anal sacs (AS) [2–6]. This protein is a noncovalent tetramer of about 35–40 kDa composed of 2 heterodimers of about 18–20 kDa linked by a noncovalent interface [7]. Each heterodimer is constituted by two covalently linked chains: chain 1 consists of 70 amino acids and chain 2 of 90- or 92-amino-acid isoforms [8–9]. Both chains are encoded by sepa-

rate genes [9] and have been assigned to 5 and 11–15 kDa, respectively, by Western blotting [10]. Additionally, chain 2 contains an N-linked oligosaccharide composed of tri-antennary glycans [11], which has been shown not to be directly involved in antibody recognition of Fel d 1 [12]. Indeed, the main antigenic region is located in the peptidic moiety of the protein [12–14] with 4 epitopes identified in recombinant chain 1, using the 6 monoclonal antibodies (mAbs) described so far [15, 16] (6F9, 3E4, 1G9, 8F3, 2H4, and 10G7). Interestingly, none of these mAbs was found to bind recombinant chain 2 [16].

Variants of Fel d 1 have long been described and have generally been assigned to a combination of glycoforms and/or truncated forms [7, 9, 11]. Particularly, chain 2 variants were reported as varying depending on source: long forms (92 amino acids) in salivary glands and short forms (90 amino acids) in skin sebaceous glands [9]. In this study, we wanted to investigate how the structural polymorphism of Fel d 1-like molecules, especially originating from various sources, can impact on the reactivity of the protein. We used mass spectrometry (MS) to characterize the panel of Fel d 1-like species, and searched a potential correlation with antibody recognition.

Methods

Cat Samples

Ten European cats from Phérosynthèse Research Institute were used throughout this study. Cats were anesthetized with intramuscular injections of 0.4 ml of Domitor® and 0.2 ml of Ketamine® (Pfizer Santé Animale, Orsay, France) and 4 sites of production collected: the cheek zone (CZ), chest area (CA), interdigital zone (IZ) and anal sacs (AS). Washing solution was distilled water containing protease inhibitors (Sigma, St-Quentin Fallavier, France). A surface of CZ about 7.5 cm² was wetted with washing solution and shaved. CA was washed with 10 ml of washing solution and rubbed with a wet compress. AS content was expressed into a dry tube and weighed. Fel d 1 was extracted overnight at 4°C under weak agitation by hair immersion or anal gland secretion dispersion in washing solution (2 ml/0.1 g of hairs, 1 ml/0.2 g of compress with harvested hairs, and 5 ml/g of anal gland secretions). IZs were washed by bathing all paws of cats in a petri plate containing 6 ml of washing solution. The interdigital spaces were carefully rubbed during the bath and further washed with 4 ml of washing solution. To remove hairs and contaminants, all these samples were centrifuged at 1,300 g for 20 min. Supernatants were divided into aliquots and kept at –20°C.

Electrophoresis and Immunoblotting

Samples (50 ng Fel d 1 in 4% SDS with or without 4 mM DTT/5 min at 100°C) were analyzed using Novex® 16% Tris-glycine gels (Invitrogen, Cergy-Pontoise, France) following the manufacturer's instructions. Gel electrophoresis in 0.1% SDS Tris-glycine running buffer (Invitrogen) was followed by transfer to a nitrocel-

lulose membrane (25 V, 100 mA for 1 h). Immunodetection was performed using the Western Breeze Chromogenic kit (Invitrogen), with a saturation step of 1 h with 1% PBST-BSA, and antibody was further used at 1 µg/ml (6F9) or 2 µg/ml (3E4, 10F7) in this buffer. The SeeBlue® Plus2 Pre-Stained Standard (Invitrogen) was used as molecular weight (MW) marker.

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry of Immunopurified Allergens

Fel d 1-like proteins were immunopurified using 6F9 mAb in Pierce Seize X Protein G Immunoprecipitation kit (Perbio Science, Brebières, France). Fractions were eluted with 40% acetonitrile, evaporated under vacuum and stored at –20°C (10-µl aliquots).

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectra were recorded on a Voyager DE-RP instrument (Applied Biosystems, Foster City, Calif., USA) in a positive-ion linear mode with delayed extraction. A saturated solution of matrix was prepared by diluting 10 mg of sinapinic acid in 500 µl of 0.1% trifluoroacetic acid in water/acetonitrile (60:40, v/v). 1-µl Fel d 1 aliquots were applied on a stainless steel sample plate for MALDI-MS. 0.7 µl of the matrix solution was subsequently added onto the spot and dried, then submitted to MALDI-TOF MS. Mass calibration was carried out on a standard solution using the monocharged ion $[M + H]^+$ at 16,951.56 Da and the double-charged ion $[M + 2H]^{2+}/2$ at 8,476.28 Da of horse apomyoglobin and the mono-charged $[M + H]^+$ of cytochrome at 12,361.96 Da. Mass spectra were then subjected to gaussian smoothing with a filter width of 19 points.

Immunoassays

The commercially available ELISA (Indoor Biotechnologies, Charlottesville, Va., USA) was used following the manufacturer's instructions to quantify Fel d 1 in all samples. The allergen standard (200 mU/ml) was cat hair extract sub-standardized using the Center for Biologics Evaluation and Research cat dander reference preparation (13.47 U/ml). Binding assays were carried out with samples containing from 0 to 120 ng/ml of Fel d 1-related material using anti-Fel d 1 mAb designated as 6F9, 3E4, and 10F7, obtained from Indoor Biotechnologies. Six formats, including the Indoor Biotechnologies kit (6F9/3E4) and 5 home-made assays, were tested to confirm all combinations with the 3 available mAbs as capture (500 ng/well; coating overnight at 4°C in pH 9.6 carbonate-bicarbonate buffer) and biotinylated tracer (50 ng/well; incubation overnight at 4°C) antibodies on 96-well microtiter plates (VWR International, Lyon, France). Washing, blocking and amplification steps were performed as described in the commercial kit. Absorbance was recorded at 405 nm. The biotin protein labeling kit was supplied by Roche (Meylan, France).

Results

Structural Polymorphism of Fel d 1 of Different Sources

Samples from a panel of 10 cats were analyzed by SDS-PAGE and Western blotting using 3 mAbs. A common pattern of several main components was observed and

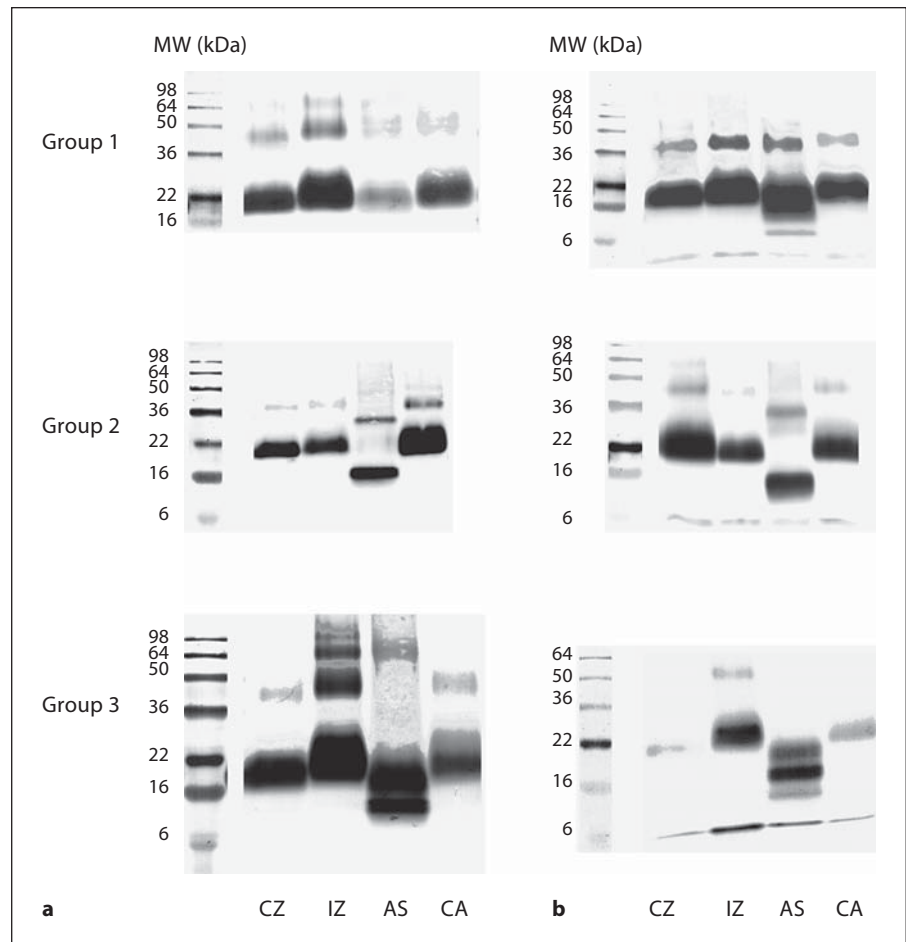


Fig. 1. Typical immunoblots of samples (approximately 50 ng Fel d 1) from various Fel d 1 sources in cats as revealed by 6F9 mAb after denaturing SDS-PAGE (**a**) and reducing and denaturing SDS-PAGE (**b**). The sources were: CZ, IZ, AS and CA.

only the most representative distribution is presented in figure 1. In all extracts, Fel d 1 material was found as a native dimer of about 20 kDa together with several polypeptides of higher and smaller apparent MWs (fig. 1a). Molecular forms of about 40 kDa could be visualized in CZ, IZ and CA of most animals, but a 30-kDa molecular form was observed in AS. The 40-kDa form was assumed to be the tetramer described in the literature [9], while the 30-kDa form may be a tetramer composed of smaller-size polypeptides. Higher-MW forms of around 64 kDa could eventually be detected in some samples of virtually all the animals except in AS, which contain molecular forms of smaller size.

Accordingly, the panel of cats involved in the study was divided into 3 groups depending on the presence of low-MW components: (1) In group 1 ($n = 1$), Fel d 1 appeared as the major component in all zones as a large and diffuse band of 19–21 kDa. (2) In group 2 ($n = 4$), Fel d 1 also appeared as a 19- to 21-kDa polypeptide in CZ, IZ

and CA while in AS an additional band of 16–17 kDa appeared to be immunologically related to Fel d 1. (3) In group 3 ($n = 5$), both the 16- to 17- and 13- to 14-kDa variants were detected in AS. In other compartments, Fel d 1 remained the main component of about 19–21 kDa. When analyzed by MS after extensive trypsin digestion, peptides from both chain 1 and chain 2 of Fel d 1 were present in all these variants (data not shown).

Most high-MW bands dissociated upon reduction (fig. 1b), but a residual 19- to 21-kDa polypeptide was observed in CZ, IZ, and CA and a 16- to 17-kDa polypeptide in AS, indicating that both types of protein maintain their reactivity to antibodies under such drastic conditions. Besides the contents, the 16- to 17- and 13- to 14-kDa variants contents, appeared quite variable among animals in AS. In contrast, all samples displayed a common 5-kDa species, presumably chain 1, and no chain 2 was found to bind any of the 3 mAbs.

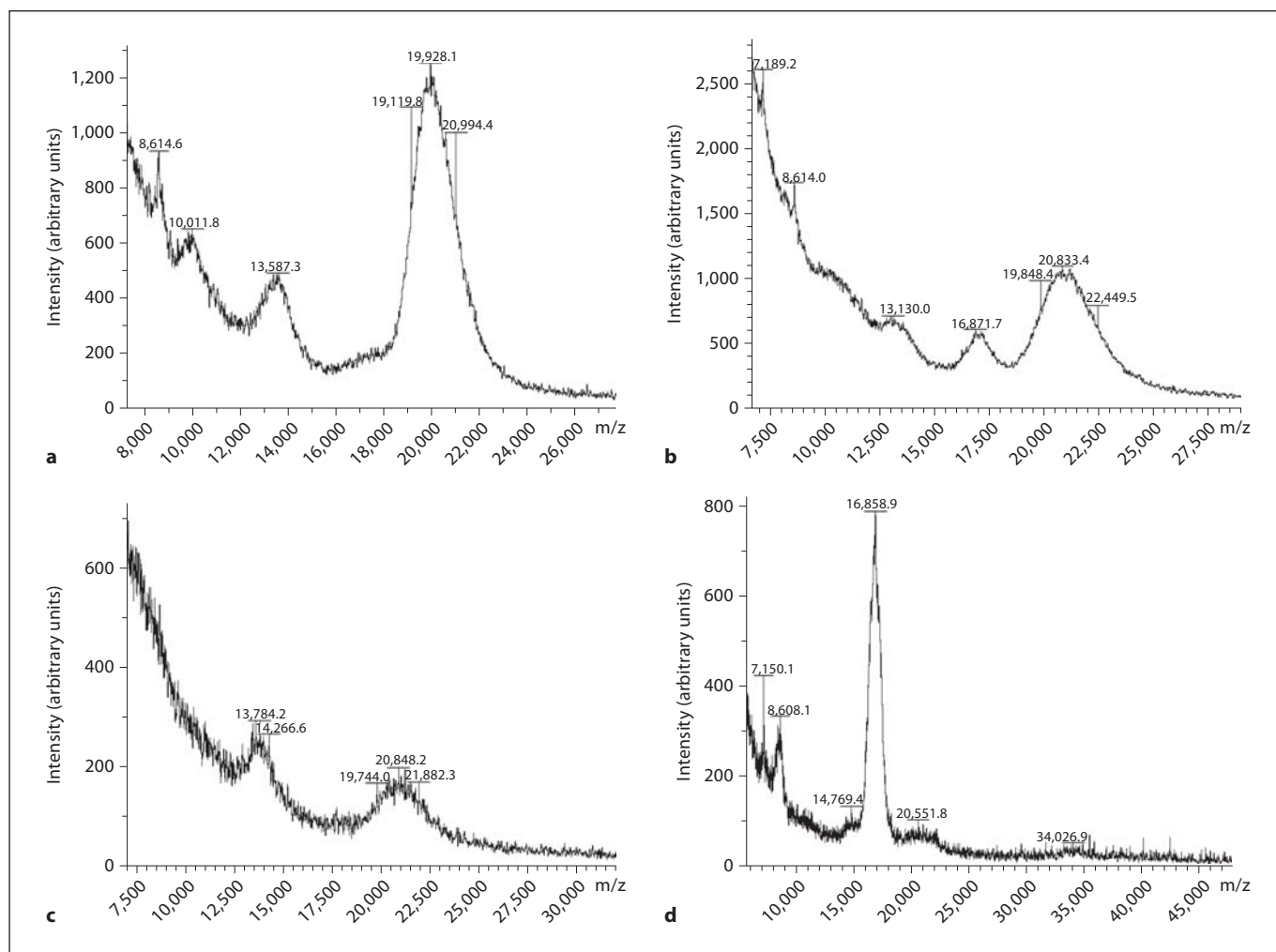


Fig. 2. MALDI-TOF MS analysis of immunopurified Fel d 1 proteins from a typical group 2 cat. **a** CZ. **b** IZ. **c** CA. **d** AS. Da should not be considered as it is the doubly charged molecular ion of its corresponding peak at 19,928.

Identification of Fel d 1 Variants by MS

To further characterize the nature of the variant pattern distributed in the various areas collected, we immunopurified Fel d 1-like proteins and subjected them to MS analysis. In preliminary tests, we found Fel d 1-like material purified with mAb 6F9, 3E4 and/or 10F7 was again quite similar (data not shown). Only the experiments with mAb 6F9 were repeated with different cats of group 2 as their extracts were the most abundant and representative. The data are summarized in figure 2 and table 1.

Figure 2 shows that Fel d 1 was present at its expected size (19,119–22,449 Da) in all compartments, but additional low-MW forms which were not observed by Western blotting could also be detected. It should be remem-

bered that in MS, in contrast to the pattern in Western blotting, peak intensity is not related to the amount of relevant polypeptide in the sample. CZ content appeared as a broad peak with a maximum at 19,928 Da and a full width at half maximum (FWHM) of 1,900 Da together with two other peaks at 13,587 and 8,614 Da (fig. 2a). In IZ (fig. 2b), Fel d 1 isoforms were distributed in a main very broad peak with a maximum at 20,833 Da (FWHM of 2,500 Da) and 3 others, with a lower signal, at 16,871, 13,130, and 8,614 Da, which were not detected either on blots. In CA (fig. 2c), Fel d 1 material consisted in a very broad peak with a maximum at 20,848 Da (FWHM of 2,100 Da) and in an additional species at 13,784 Da (FWHM of 1,000 Da). In striking contrast, Fel d 1 from

Table 1. Molecular mass of Fel d 1 protein under nonreducing and reducing conditions for the four studied areas of a typical cat from group 2

	Molecular mass observed (peak top), Da			
	CZ	IZ	AS	CA
Nonreducing conditions	19,928	20,833	20,552	20,848
	13,587	16,872	16,859	13,784
	8,615	13,130	14,769	
Reducing conditions		8,614	8,608	
		7,189	7,150	
	13,399	13,064	8,607	13,614
	8,109	8,219	8,218	8,994
	5,378	5,235	8,039	5,207
			5,382	4,901

Major peaks are in bold type. According to the literature [10], the calculated molecular mass is 7,869.0 Da for chain 1 and 9,999.5 Da for chain 2. Nonglycosylated Fel d 1 is about 17,950 Da on average. Glycans range from approximately 1,631 to 2,869 Da and the glycosylated Fel d 1 was thus assumed to range from approximately 19,581 to 20,819 Da.

AS (fig. 2d) displayed a peak with a maximum at 16,859 Da (FWHM of 1,000) and three other peaks with a lower signal at 20,551, 14,769 and 8,600 Da. These molecular forms are in agreement with the forms detected by SDS-PAGE, except for the peak at 8,600 Da. Assuming the intact Fel d 1 dimer has a well-accepted size of 19–21 kDa, the marked width of related peaks exhibited intrinsic microheterogeneity likely to be caused by N-linked glycosylation [11], whatever the site of production. These results thus confirmed the strong heterogeneity of the allergen in all compartments and further revealed additional low-MW forms which could not be detected under more denaturing conditions such as Western blotting. Interestingly, these fragments were of reproducible size in all extracts.

These findings indicated that the allergen may undergo degradation or exist as truncated variants [9]. Fel d 1 material of about 13–14 and 16–17 kDa was intriguing as it could result from deletion of chain 1, chain 2 and/or both. To further identify these polypeptides, we performed an MS analysis after reduction of the immunopurified sample (table 1). In most areas, a polypeptide of 5 kDa was identified and was attributed to chain 1 by fingerprinting studies (data not shown). The 8-kDa polypeptide identified upon reduction was similarly assigned to chain 2. A molecular form of 13 kDa was still present and could be either nonreduced dimers resulting from

the cross-linked truncated chain 1 (5 kDa) and truncated chain 2 (8 kDa) or from the intact chain 2 (13 kDa), as observed by van Milligen et al. [10]. However, the 13-kDa variant could not be unambiguously characterized by mass fingerprinting (data not shown). From these findings, we deduced that Fel d 1 is likely truncated in chain 1 and/or chain 2 to produce low-MW products that are still associated as a dimer through disulfide bonding. These short variants of 16–17 and 13–14 kDa will be further referred to as core fragments in subsequent studies. Of note, these components were consistently identified in the panel and among the three groups of animals (data not shown) and should thus be considered as reproducible truncated forms of the allergen in the main sites of production in the cat.

Immunological Polymorphism of Fel d 1 from Different Sources

Considering that truncation of Fel d 1 may occur in each animal, a key question was to understand how this process can affect the immunological behavior of the protein. We analyzed antibody recognition in all extracts where core fragments have been defined. In figure 3, we selected typical dose-dependent curves of the various Fel d 1 samples using two distinct formats. Figure 3a shows 6F9/3E4 antibody recognition was similar whatever the origins of Fel d 1 with close to maximal and half-maximal binding capacities, except for AS for which antibody recognition was reduced by 50%. In figure 3b, the use of the 10F7 mAb as tracer antibody dramatically changed recognition of Fel d 1 material. Although the maximal binding capacities of the samples remained roughly similar, half-maximal binding of all samples was markedly different. There was a 3-fold difference between the standard and the less reactive sample (AS). CA and CZ Fel d 1 displayed similar half-maximal binding while IZ Fel d 1 displayed intermediate recognition between CZ/CA/standard and AS. This indicated that a variable amount in core fragments may alter the Fel d 1 content value of a sample.

To understand how degraded Fel d 1 reacts in immunoassays, we analyzed the various extracts in all the 6 formats which may be constructed with a panel of 3 mAbs. Figure 4a shows that formats and samples displayed similar maximal binding capacity except when the 10F7 mAb was used as capture antibody. Most formats were thus able to estimate the same amount of Fel d 1 in all samples as in the standard at high antigen concentrations. However, each format reacted very differently in the presence of limited amounts of antigen (fig. 4b), indi-

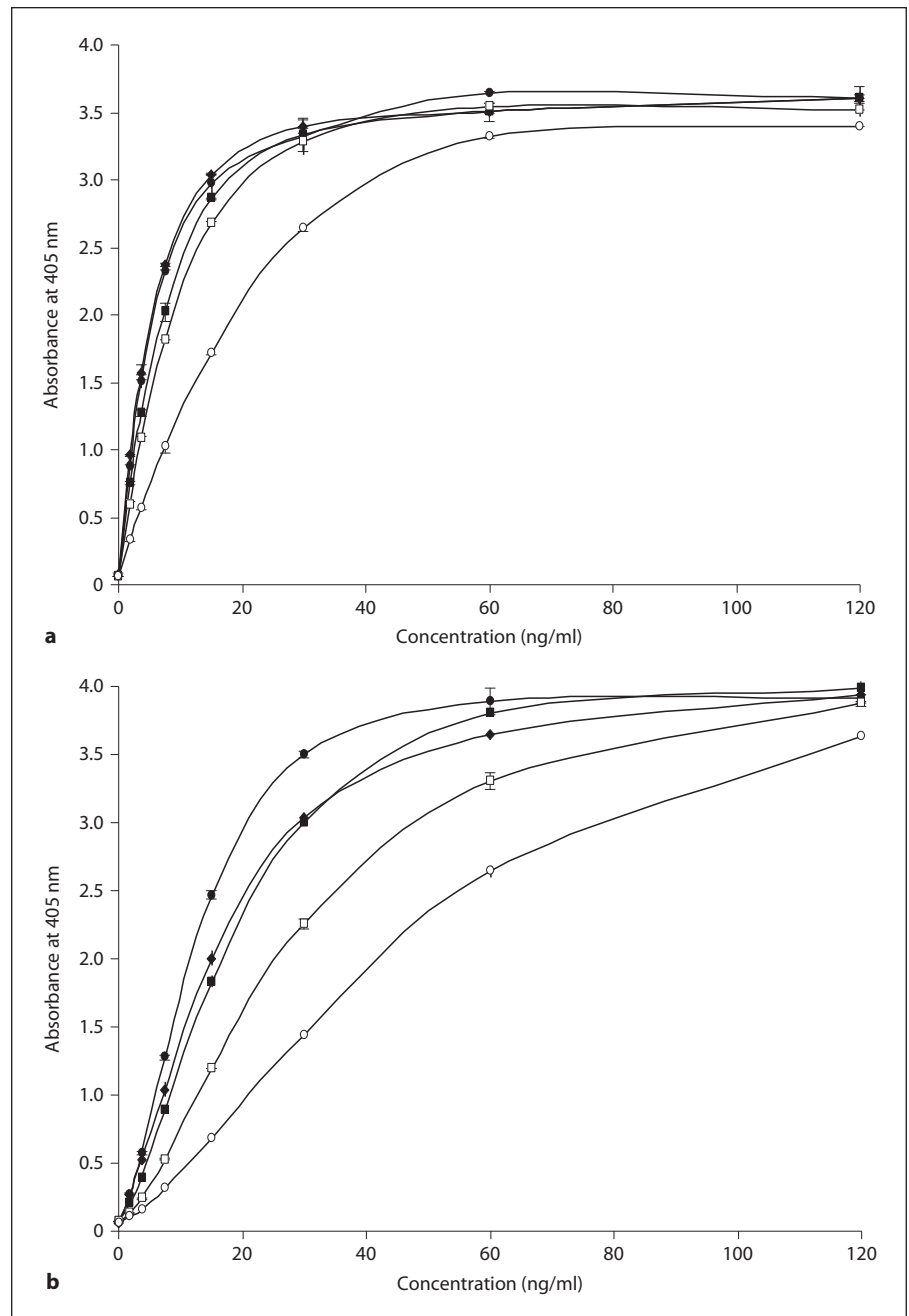
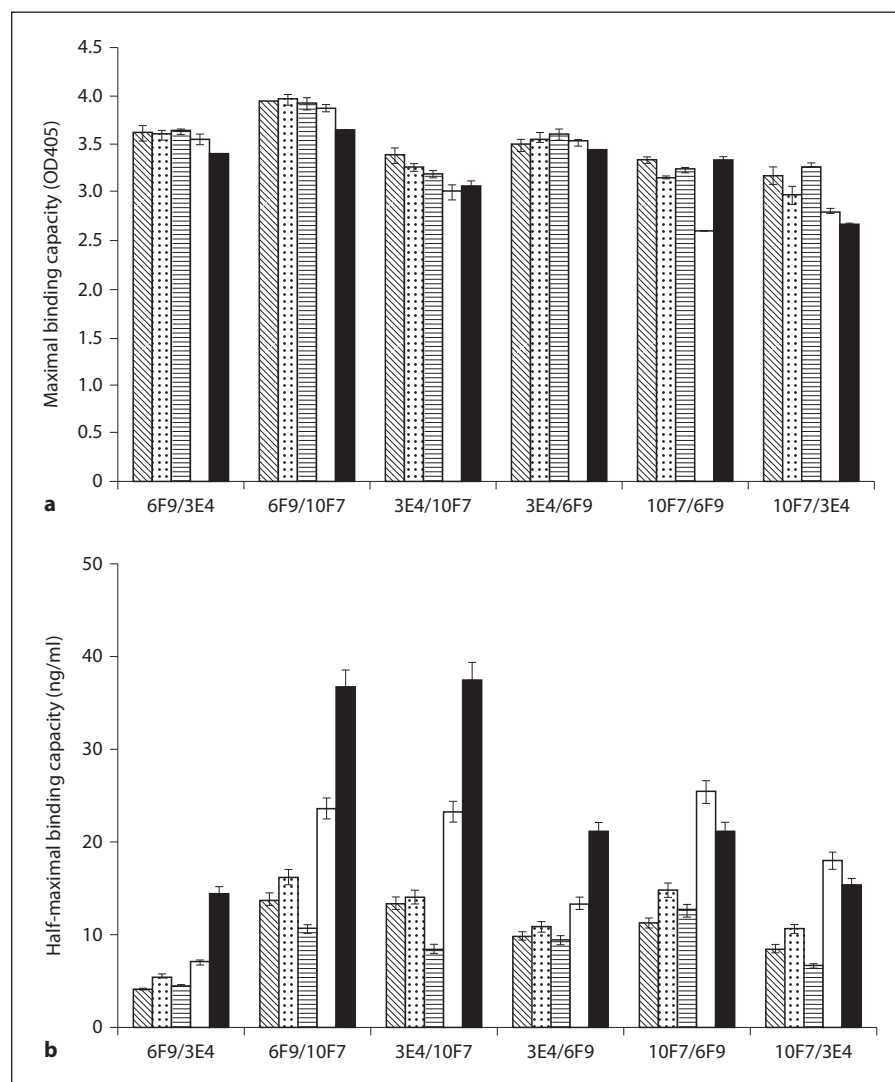


Fig. 3. Dose-dependent curves at equilibrium of the 6F9/3E4 format (a) and the 6F9/10F7 format (b) using standard (◆), CA Fel d 1 (■), CZ Fel d 1 (●), IZ Fel d 1 (□) or AS Fel d 1 (○). Assays were done in triplicate.

cating differences in antibody recognition within formats as well as among samples. Strikingly, Fel d 1-like material from AS was by far the less reactive, with up to a 2-fold increase in the 3E4/6F9, 10F7/6F9 and 10F7/3E4 formats and a 3-fold increase in the 6F9/3E4, 6F9/10F7 and 3E4/10F7 formats compared to the standard. Recognition of Fel d 1 content of IZ was also significantly impaired (1.5- to 2-fold increase in half-maximal binding

capacity) compared to the standard in 10F7 mAb-based formats. In all formats, the half-maximal binding capacity of Fel d 1 from CA was the closest to the standard. CZ content was slightly more reactive in some formats (6F9/10F7, 3E4/10F7, 10F7/3E4). The format which displayed optimal antibody recognition for all the samples appeared to be based on 6F9 and 3E4 mAbs. Altogether, these data indicated that the extracts differ in immuno-

Fig. 4. Antibody recognition of Fel d 1 from different allergen sources in 6 various formats of ELISA assays at equilibrium. **a** Maximal binding capacity. **b** Half-maximal binding capacity. ▨ = Standard; ▩ = CA Fel d 1; ▧ = CZ Fel d 1; □ = IZ Fel d 1; ■ = AS Fel d 1. Samples were tested in triplicate.



reactivity, likely due to a variable ratio of core fragments to intact Fel d 1.

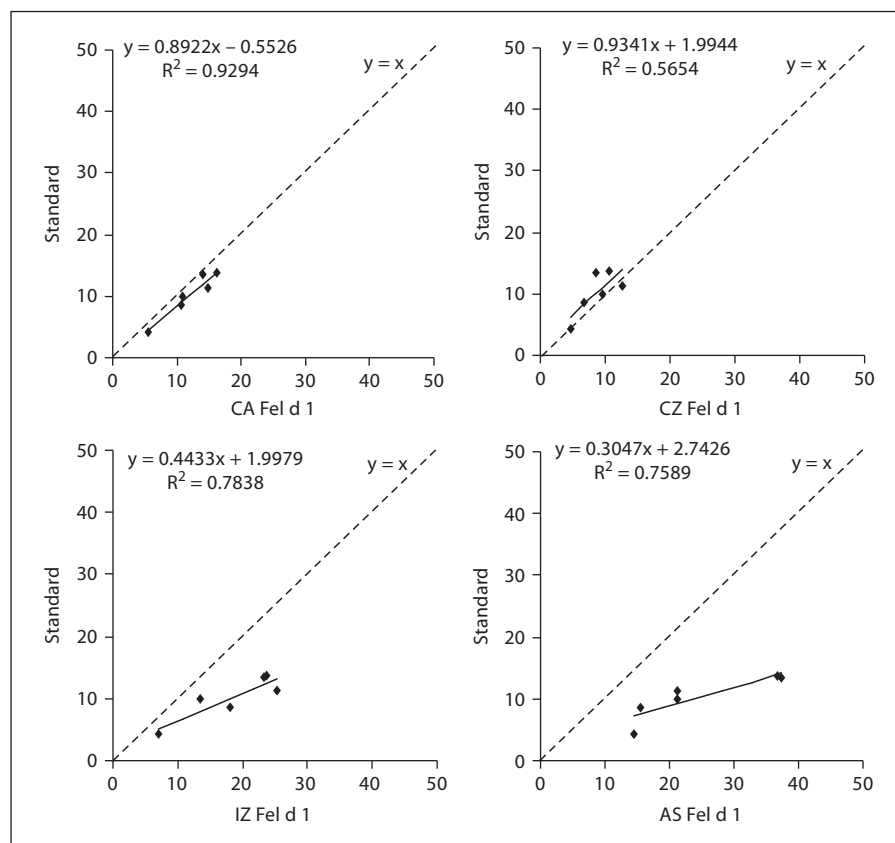
To finally estimate to what extent core fragments interfere with allergen measurement and potentially decrease accuracy in extracts from different sources, we plotted half-maximal binding capacity of Fel d 1 from different sources against the standard in the 6 formats (fig. 5). CA and CZ Fel d 1 indeed matched well with the standard, whereas IZ and AS Fel d 1 were far less reactive. The slopes of linear regression were of 0.8922 and 0.9341, respectively, for CA Fel d 1 and CZ Fel d 1, while the slopes of linear regression of IZ Fel d 1 and AS Fel d 1 were of 0.4433 and 0.3047, confirming that the material present in these areas widely differed from the two others. Coefficients of correlation (R^2) from linear regression

analysis for CA samples were about 0.9, indicating a rather homogeneous recognition of this form among the 6 formats. In contrast, CZ, IZ, and AS samples displayed values highly dependent on the assay, with R^2 of 0.5654, 0.7838, and 0.7589, respectively.

Discussion

This study further confirmed the well-known polymorphism of Fel d 1 and demonstrated that it contains core fragments that are consistently present in most cats and in the main anatomical sites of production. In all extracts, essentially two truncated dimers of 13–14 and 16–17 kDa, respectively, were identified in combination with

Fig. 5. Correlation of half-maximal binding capacity between the standard and different allergen sources in the 6 formats tested. Equations and correlation coefficients of the linear regressions are indicated in the graphs. It is generally assumed that identity of epitope expression between two preparations generates a slope value of 1 ($y = x$).



intact Fel d 1 except in AS where they represent the most prominent if not unique molecular forms. These core fragments were reproducibly found in all animals but in rather variable amounts. Interestingly, we also show that antibody recognition did not necessarily reflect the extent of allergen truncation. Recognition of 6F9 and 3E4 mAbs was not dramatically affected by a variable content in core fragments, and as a result, intact and truncated forms of Fel d 1 were both fully detected in this assay. Conversely, loss of 10F7 recognition of Fel d 1 material in AS and IZ indicated that fragments indeed displayed an altered conformation compared to intact Fel d 1. Such immunological behavior suggested that depending on the assay, allergen measurements may vary and do not accurately reflect the Fel d 1 content.

In full agreement with previous reports [10, 11], immunoblot experiments confirmed that the allergen was present in each of the sampled areas as tetrameric and dimeric intact Fel d 1-related proteins and also as a wide array of species of high and low MW and therefore exists as a complex mixture of immunoreactive variants. The relative distribution of these components clearly differed

among areas but not so much among animals. High-MW forms (approximately 40 and 64 kDa) were assigned to oligomers as they were reactive to three distinct anti-Fel d 1 antibodies and converted into the 19- to 21-kDa heterodimer upon reduction. At least one peptide from each Fel d 1 chain was found in these molecules using peptide fingerprinting studies (data not shown), confirming they were tetramers and possibly hexamers. However, aggregates may also be present as described in previous studies [10] and can result from random association of dimers through hydrophobic interactions. Such artifactual interactions may occur in the animal as well as during sampling and storage of the extracts, delivering samples which may be resistant to denaturation as known for many other proteins. Such aggregates are especially difficult to evaluate since in these artifactual materials, too remote epitopes may not necessarily be present to allow proper quantification by ELISA.

In most compartments, shortening of chain 1 and chain 2 appeared quite extensive and could be up to 20 amino acids in each subunit, leading fragments ranging from 16 to 17 and 13 to 14 kDa. Based on MS analysis, es-

entially internal peptides could be identified in both chains, leading to disulfide bonded core fragments still capable of maintaining a tetrameric state as an approximately 30-kDa molecular form. C-terminal and N-terminal peptides could never be repeatedly identified in peptide fingerprints, possibly because they may be degraded by exoproteases at the anatomical site of production or when cats lick themselves as saliva does contain active proteases. The physiological compartments where this potential degradation is optimal were found to be IZ and particularly AS, where these low-MW polypeptides may be virtually the only component in many cats. Alternatively, an active bacterial life has been described in AS [17] and this probably contributes to major proteolytic activity. Variability in the content of Fel d 1 fragments in all the compartments may thus be due to cross-contamination caused by licking and/or by accumulation of any secretions under the paws. Their transport can be facilitated by the saliva of the animal which helps propagating core fragments all over the body when the cat licks itself. In addition, the AS secretion is part of the cat's defense system and is widely used for marking or alarming purposes [18]. This phenomenon which reflects the emotional status of the animal may also noticeably impact on the variable content in Fel d 1 core fragments spread out by the cat. This hypothesis is currently under investigation by some of us.

In this study, we were able to show that the structural polymorphism of Fel d 1 has an unexpectedly low impact on the immunoreactivity of the protein. Both Fel d 1 and related truncated molecular forms were found to be reactive to antibodies in both Western blots and immunoassays throughout this study, even though chain 1 may be largely truncated at both ends. Earlier reports have shown that 6F9, 3E4, and 10F7 mAbs essentially target chain 1 and bind native as well as reduced forms of this subunit [10, 16]. Our findings show that core fragments still co-express the 3 epitopes in both tetrameric and dimeric forms and are therefore fully immunoreactive. The 10F7 epitope was found to be the most sensitive to changes in conformation and is poorly expressed in core fragments. It has been previously reported that there may be a partially cryptic epitope in Fel d 1 [19], which is not consistently recognized by antibodies according to its origin, especially in anal gland secretions, and to the methods of sample conservation. This is in good agreement with a substantial decrease in 10F7 recognition observed in AS and IZ extracts. In contrast, the 6F9/3E4 format was found to efficiently detect all forms of Fel d 1 from any origin. Antibody recognition was never abolished but

was decreased by a factor of 2 in IZ and AS samples. This may lead to a gradual misestimation of the allergen in a given sample in case of changes in the Fel d 1 molecular forms. Increasing the content in core fragments will cause diminished antibody recognition as they will compete with intact Fel d 1 and finally decrease the accuracy of Fel d 1 measurements [Bienboire-Frosini et al., in preparation]. Indeed, large variations in the values assigned to the allergen content of extracts have been well documented [20] and are being actively investigated for allergen standardization [21–23]. As suggested by some authors [23, 24], broad recognition of Fel d 1 in the 6F9/3E4 format may certainly be an advantage provided that the calibrator can display the appropriate heterogeneity. There is currently an active debate for developing certified reference materials based on natural and/or recombinant Fel d 1 [23–25]. Our findings show that a common pattern of variants can be defined within a group of cat variables as a function of the origin of the sample. If such a preparation can be properly isolated or engineered and validated as a universal Fel d 1 allergen source, it will be of potential help for standardizing cat extracts used in diagnosis and immunotherapy.

An intriguing and important question raised by the presence of core fragments is how they relate to the allergenicity of the Fel d 1 protein. Human IgE have been demonstrated to strongly react with Fel d 1 chain 1 as well as the different MW fragments related to Fel d 1, including the lower MW polypeptides [10]. Indeed, some authors [26] have shown that most of T cell reactivity is located in chain 1, particularly in the peptides encompassing the 9–55 and 56–69 residues. Based on MS, we found that the median region is conserved in core fragments of AS (data not shown). It will be worth determining how patients sensitized to cats react to core fragments compared to intact Fel d 1. Work is ongoing to investigate IgE binding to core fragments from both animals and house dust samples to see if they positively correlate with the sensitivity of allergic patients.

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