

Fel d I levels in cat anal glands

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Summary

Background Major cat allergen *Fel d* I is produced consistently by skin and by sebaceous glands before being spread on the fur.

Objective Since cats have tubular anal glands secreting sebum, proteins and lipids, we looked at the possible presence of *Fel d* I in these secretions and compared the levels found to those already reported in other cat tissues or secretions.

Methods Thirty-seven cats were studied. *Fel d* I dosage in the anal sacs' secretions was performed using an enzyme linked immunosorbent assay (ELISA) method and total protein evaluation by the Bradford's method.

Results The geometric mean *Fel d* I concentration was 41 U/g secretion which represents 3.4% of the total protein levels. This amount is the highest ever reported in cat tissues or secretions.

Conclusion The close association of *Fel d* I protein with skin sebaceous glands and anal sacs both with holocrine function and lipids' secretions in one hand, and the homology of chain I of *Fel d* I with some steroid-binding proteins in other hand, suggest a possible physiological role for *Fel d* I in the regulation of lipids on skin and cat fur.

Keywords: cat, *Fel d* I, cat anal sac, cat sebaceous glands, cat fur

Clinical and Experimental Allergy, Vol. 26, pp. 178–180. Submitted 7 April 1995; revised 21 June 1995; accepted 8 September 1995.

Introduction

Inhalation of animal proteins may cause an immediate-type reaction in atopic persons. Amongst domestic animals, cats and dogs are the most often involved in allergic diseases [1]. Studies performed by Ohman *et al.* in 1977 [2] demonstrated that IgE antibodies in patients allergic to cats were for a large part directed against the cat allergen *Fel d* I. The biological role of *Fel d* I for the cat itself still remains unknown. This protein is a heterodimeric glycoprotein with two 17 kDa subunits each of which is composed of two chains: no I, MW 7000 and no II, MW 10 000 [3]. Several studies demonstrated saliva to be a major source of the protein and according to general opinion it is said that the presence of *Fel d* I in pelt is due to deposition of the allergen during grooming.

However, in 1985 Bartholomé *et al.* [4] suggested that *Fel d* I might originate from sebaceous glands connected to hair roots. Production of *Fel d* I by skin and by sebaceous glands in the latter was confirmed by studies done by our group [5,6]. Furthermore, it was recently demonstrated that *Fel d* I concentrations on the skin are under hormonal control in parallel with the sebaceous cells and glands volume [7]. Cats have tubular anal glands which have been described in histochemical study as structures of apocrine and holocrine glands with a large amount of sebaceous glands and secretion of sebum, proteins, and lipids [10]. Thus, we looked at the possible presence of *Fel d* I in the secretions from anal glands.

Materials and methods

Thirty-seven adult domestic cats (12 females and 25 males) were studied. Secretions from anal sacs were

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collected by pressing the glands into plastic tubes in a way that no contaminations from faeces nor genital secretions could occur and stored at -80°C . *Fel d I* dosage in the samples was performed using a two-site monoclonal antibody (MoAb)-based enzyme-linked immunosorbent assay (ELISA) with *Fel d I*-specific MoAb provided by M. D. Chapman and T. A. E. Platts-Mills (Charlottesville, VA, USA). Maxisorp F16, flatbottom microtitre plates (Nunc Immuno module, Polyabo Paul Block et Cie, Strasbourg, France), were coated overnight with 1 mg per well of 6F9 MoAb in bicarbonate buffer 0.1 M pH 9.6, at 4°C . The plates were then washed twice with phosphate-buffered saline (PBS) 0.05% Tween 20 (PBS-T), pH 7.4 n 300 mL per well and incubated for 30 min with 100 mL per well and incubated for 30 min with 100 mL per well PBS-T-bovine serum albumin at room temperature. After two more washes with PBS-T, either *Fel d I* reference solution (cat reference E3, H. Baer, Office of Biologics and Research Resources, Bethesda, MD, USA) or diluted samples were added to wells (100 mL per well). Doubling dilutions of cat E3 were used to form a control curve from 46.5 to 0.7 mU/mL. Four dilutions of each sample (from 1/5 1/50 1/500 1/1000) were assayed twice. After a 1 h-incubation period at room temperature, plates were washed four times with PBS-T. Biotinylated 3E4 MoAb was added (100 mL per well of a 1/1000 PBS-T-bovine serum albumin dilution), and plates were incubated for 1 h and then washed four times with PBS-T. Streptavidin-peroxidase (Sigma Chimie Sarl, La Verpillière, France) was added (100 mL per well of a 1/1000 PBS dilution or approximately 43×10^{-6} units per well) and plates were incubated for 30 min and washed four times with PBS-T. The substrate orthophenylenediamine (Abbott Laboratories, Rungis, France) was added (25.6 mg in 10 mL of solvent; 100 mL per well), and plates were incubated for 20 min in darkness. The optical density of each well was measured at 405 nm (Titertek Multiscan Plus MK II, Flow Laboratories, Efflaboy, Finland) and compared with values on the reference scale. Results were calculated as the mean of two wells at the same dilution. The lowest allergen concentration detected was about

Table 1. *Fel d I* and protein concentrations (geometric means and ranges) in anal sacs' secretions

	<i>n</i>	<i>Fel d I</i> U/g	Proteins mg/g
Males	12	39.2 (2.2–339)	4.8 (0.8–10.6)
Females	25	46.3 (9.8–17.6)	5.1 (3.1–9.5)
Males & females	37	41.4 (2.2–339)	4.9 (0.8–10.6)

Table 2. *Fel d I* levels found in cat tissues or secretions reported in the literature

Tissues/secretions	<i>Fel d I</i> concentration U/g or U/mL	Reference
Milk	0.33	9
Saliva	4–7	9
	1–8	8
Lacrimal fluid	7–14	9
Sublingual gland	0.61	9
	0.48	8
Submandibular gland	0.04–0.05	9
Parotid gland	0.07–0.21	9
Lacrimal gland	0.39–0.78	9
Pelt	8.56	9

0.7 U/mL. Total protein was assayed by the Bradford's method. One unit of *Fel d I* was considered equal to $4 \mu\text{g}$ protein.

Results

The concentrations of *Fel d I* in anal sacs' secretions are listed in Table 1. The geometric mean *Fel d I* concentration (males and females) was 41 U/g secretion with a range from 2 to 339 U/g. This represents 3.4% of the total protein level. There were no statistical difference between males and females but a high cat to cat variability. Possible intracat variability of *Fel d I* production cannot also be excluded.

Discussion

The amount of *Fel d I* found in cat anal secretions is the highest ever reported in cat tissues or secretions. In the literature, *Fel d I* was not detectable in serum or in urine [8]. Table 2 shows the *Fel d I* levels from other studies. Less than 1 U/mL or /g was found in milk, parotid, submandibular, sublingual lacrimal glands [9]. The highest levels previously found were demonstrated in saliva (4–7 U/mL) in lacrimal fluid (7–14 U/mL) and cat pelt (8.6 U/g [8,9]. The huge amount of *Fel d I* in cat anal sacs' secretions may be explained by the structure of the anal glands with a dominant holocrine secretion [10]. Thus, besides skin and saliva, *Fel d I* on cat fur may also originate from anal glands. On the other hand, chain I of *Fel d I* shows some homology to steroid binding proteins such as rabbit and brown hair uteroglobulin, rat prostatic chain precursor protein Cl rat polychlorinated biphenyl binding protein precursor and mouse salivary androgen-binding protein alpha subunit [11]. At this time no

other homology with other active agents and no enzymatic function of *Fel d I* were described. The close association of *Fel d I* protein with skin sebaceous glands and anal sacs all with holocrine function and lipids secretions suggest a possible physiological role for *Fel d I* in the regulation of lipids on skin and cat fur. *Fel d I* might also play a role in sex and species recognition by binding specific lipids.

References

- 1 Sarsfield JK, Boyle AG, Rowell EM, Moriarty SC. Pets sensitivities in asthmatic children. *Arch Dis Child* 1976; 51:186.
- 2 Ohman JL, Rendall S, Lowell FC. IgE antibody to cat allergens in allergic population. *J Allergy Clin Immunol* 1977; 60:317–23.
- 3 Morgenstern JP, Griffith I, Brauer AW et al. Amino acid sequence of *Fel d I*, the major allergen of domestic cat: protein sequence analysis and cDNA cloning. *Proc Natl Acad Sci* 1991; 88:9690–4.
- 4 Bartholomé K, Kissler W, Baer H, Kopietz-Schulte E, Wahn U. Where does cat allergen I come from? *J Allergy Clin Immunol* 1985; 76:503–6.
- 5 Dabrowski A, Van der Brempt X, Soler M et al. Cat skin as an important source of *Fel d I* allergen. *J Allergy Clin Immunol* 1990; 86:462–5.
- 6 Charpin C, Mata P, Charpin D et al. *Fel d I* allergen distribution in cat fur and skin. *J Allergy Clin Immunol* 1991; 88:77–82.
- 7 Charpin C, Zielonka T, Charpin D et al. Effects of castration and testosterone on *Fel d I* production by sebaceous glands of male cats. II Morphometric assessment. *Clin Exp Allergy* 1994; 24:1174–8.
- 8 Brown Ph, Leiterman K, Ohman JL. Distribution of cat allergen I in cat tissues and fluids. *Int Archs Allergy Appl Immunol* 1984; 74:67–70.
- 9 van Milligen F, Vroom T, Aalberse R. Presence of *Felis domesticus* allergen I in the cat's salivary and lacrimal glands. The human antibody response to the cat allergen. F. Van Milligen Thesis, 1993.
- 10 Flachsbarth M. Functional morphology of anal sacs and their glands of domestic cats. Thesis, 1990.
- 11 Karn R. The mouse salivary androgen-binding protein (ABP) alpha subunit closely resembles chain 1 of the cat allergen *Fel d I*. *Biochem Gen* 1994; 32:271–7.

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