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Presence of *Felis domesticus* Allergen I in the Cat's Salivary and Lacrimal Glands

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Abstract. Most of the IgE response in persons allergic to cats is directed against the cat allergen Fel d I (*Felis domesticus* allergen I). Although the presence of Fel d I in saliva has been demonstrated, the source of Fel d I is not completely known. We measured Fel d I concentrations in the cat's lacrimal and salivary glands and in the excretes lacrimal fluid, milk and saliva. The concentration of Fel d I, determined by a radioimmunoassay, in the lacrimal gland was 390–780 mU/g tissue and in the sublingual gland 610 mU/g tissue. In the parotid gland and especially the submandibular gland only low concentrations were found, respectively 70–210 and 40–50 mU/g tissue. In lacrimal fluid 6.8–14 U/ml Fel d I was detected, which is comparable to saliva, 4.2–7.0 U/ml, whereas in cat's milk only 0.33 U/ml Fel d I was found. Immunohistochemical studies with monoclonal antibody directed against Fel d I (anti-Fel d I) showed the presence of Fel d I in the serous cells of the lacrimal gland. Thus, our results demonstrate that in particular the lacrimal gland might be a useful Fel d I source.

Introduction

In 20–30% of patients with asthma and/or rhinitis, sensitization to cat allergens is found [1, 2]. Studies performed by Ohman et al. [3] in 1977 and Lowenstein et al. [4] in 1985 demonstrated that IgE antibodies in those patients were for a large part directed against the cat allergen Fel d I.¹ Quantitative studies by De Groot et al. [5] with polyclonal and monoclonal antibodies against Fel d I confirmed this: after Fel d I depletion of cat dander extract >75% of the patients showed a >75% reduction of the IgE response in the RAST.

The biological role of Fel d I for the cat itself still remains unknown. The 33 N-terminal amino acid residues were sequenced by Chapman et al. [6], and showed no homology with other known protein sequences. Several studies demonstrate saliva to be a major source of the protein [7–9], and the presence of

Fel d I in the pelt is partly due to deposition of the allergen during grooming. Bartholomé et al. [8] showed by immunohistochemical studies the presence of Fel d I in the mucous sublingual gland. In these studies, however, they also showed that the sebaceous glands, connected to hair roots, are important allergen sources. Production of Fel d I in the latter was confirmed by studies done by Dabrowski et al. [9].

Brown et al. [10] studied the distribution of Fel d I in other cat tissues and fluids by quantitative immunoelectrophoresis and found high concentrations of Fel d I in cat pelt and brain extracts, respectively 8.56 and 1.04 mU/mg tissue. Fel d I was not detectable in serum [7] or in urine [7, 10].

Until now no data on the presence of Fel d I in body secretes other than saliva are available. The aim of this study was to investigate the presence of Fel d I in the cat's lacrimal and salivary glands and find a good Fel d I source for biosynthetic experiments and for isolating the mRNA of Fel d I which can be used for cloning the Fel d I cDNA. The glands and their excretes were studied by immunohistochemical means and a RIA for the presence of this allergen. Also, the concentration of Fel d I in another excrete, cat's milk, was studied.

¹ Abbreviations: BSA = bovine serum albumin; Fel d I = *Felis domesticus* allergen I; MAb = monoclonal antibody; MAb CLB-Fd1a = monoclonal antibody directed against Fel d I; PBS = phosphate-buffered saline, pH 7.4; RAST = radioallergosorbent test; RIA = radioimmunoassay; TBS = Tris-buffered saline, pH 7.6; U = unit (1 U Fel d I = 2–4 µg).

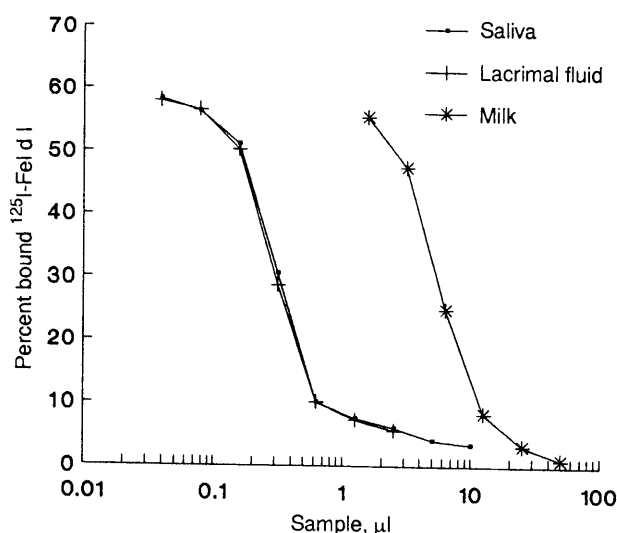


Fig. 1. Dose-response curves for saliva, lacrimal fluid and milk. 50 µl of a 1:1,000 dilution of rabbit antiserum directed against Fel d I was preincubated with 50 µl sample dilution. After 2 h 0.5 ml protein A Sepharose (1 mg/ml) and ^{125}I -Fel d I were added and an incubation overnight followed. The Sepharose was washed and the percentage bound ^{125}I -Fel d I was measured.

Table 1. Fel d I concentration and total protein concentration in the cat's salivary and lacrimal glands and in some excretory products: the glands of 2 cats were studied, only the sublingual gland was tested once

Gland/excrete	Fel d I concentration		Total protein concentration	
	mU/g	U/ml	mg/g	mg/ml
Parotid gland	70-210		70	
Submandibular gland	40-50		40	
Sublingual gland	610		33	
Lacrimal gland	390-780		12	
Saliva		4-7		0.4
Lacrimal fluid		7-14		6
Milk		0.33		54

Materials and Methods

Animals

Salivary and lacrimal glands were obtained from 2 adult female cats after being used for experiments at the Biochemical Laboratory of The Netherlands Ophthalmic Research Institute in Amsterdam. Within 1 h postmortem, the glands were removed and stored at -70°C . Tissue extracts (3%) were made by adding PBS and homogenizing 4×1 min by a Sorvall Omnimixer (16,000 rpm). Insoluble matter was separated from the extract by centrifugation for 10 min at 12,000 g.

To obtain cat's milk the kittens were removed from their mother. After 12 h the mother cat was milked by hand and the milk was collected in a funnel. Fat was removed by adding 50% (v/v) Freon TF (Hoekloos, Schiedam, The Netherlands). After thorough mixing the milk was centrifuged for 10 min at 1,500 g, the upper water fraction was collected and dialyzed overnight against PBS.

To obtain saliva the animal was anesthetized with 0.6 ml aescoket intramuscularly (100 mg/ml ketamine HCl; Harlan CPB, Austerlitz, The Netherlands). To obtain a clear fluid, saliva was centrifuged through a 3-cm layer of Sephadex G-25 (Pharmacia AB, Laboratory Separation Division, Uppsala, Sweden).

Lacrimal fluid was collected (without stimulation) with a hypodermic syringe after anesthetizing the cat with 0.6 ml aescoket intramuscularly.

Monoclonal Antibodies

MAB anti-Fel d I, CLB-FdIa [5], was purified from mouse ascitic fluid using protein A Sepharose CL-4B (Pharmacia Fine Chemicals, Uppsala, Sweden), according to the manufacturer's instructions.

RIA Procedure: Fel d I Assay

The Fel d I assay was performed by a competition RIA described by De Groot et al. [5], with rabbit antiserum directed against cat dander from ALK (Allergologisk Laboratorium, Copenhagen, Denmark) and ^{125}I -Fel d I (specific activity 0.125 mCi/U Fel d I).

Immunohistochemical Methods

The indirect immunoalkaline phosphatase method has been described elsewhere [11]. Briefly, tissue specimens of normal feline salivary and lacrimal glands were snap-frozen in liquid nitrogen. 6-µm frozen sections were cut, air-dried and fixed in acetone for 10 min. Fixation and all subsequent washes and incubations were performed at room temperature. After fixation, sections were washed for 5 min in three changes of PBS, incubated for 30 min with protein A purified MAB CLB-FdIa, 0.93 mg/ml, in 1% BSA in PBS, washed again in three changes of PBS and incubated for 15 min in alkaline phosphatase-conjugated rabbit anti-mouse immunoglobulin (Dakopatts D314, Glostrup, Denmark), diluted 1:20 in 1% BSA in PBS. To this diluted antiserum, 10% normal cat serum was added to reduce background staining. After three changes of TBS, alkaline phosphatase activity was visualized according to the slightly modified method of Li et al. [12] by a 30-min incubation in the dark with new fuchsin staining mixture. This mixture is made as follows: a 4% w/v solution of NaNO_2 in H_2O is thoroughly mixed with an equal volume of a 4% w/v volume of new fuchsin (Chroma 1B467, Stuttgart, FRG) in 2 M HCl (solution A). Naphthol AS-MX phosphate (N-5000; Sigma Chemical Co., St. Louis, Mo.) and levamisole (L-9756; Sigma) are dissolved in dimethylformamide at 0.9% and 0.75% w/v respectively and diluted subsequently 30 times in 0.2 M Tris-HCl, pH 8.0 (solution B). The final staining mixture, to be used freshly, is made by diluting solution A 200 times into solution B, adjusting the pH to 8.0 and filtering. After staining, sections were washed in distilled water for 5 min, counterstained in Mayer's hematoxylin, washed in running tap water for 5 min and mounted in glycerin-gelatin.

In a control experiment, the anti-Fel d I activity was reduced by incubating MAB CLB-FdIa (186 µg MAB) with 40 mg CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals) to which 160 µl

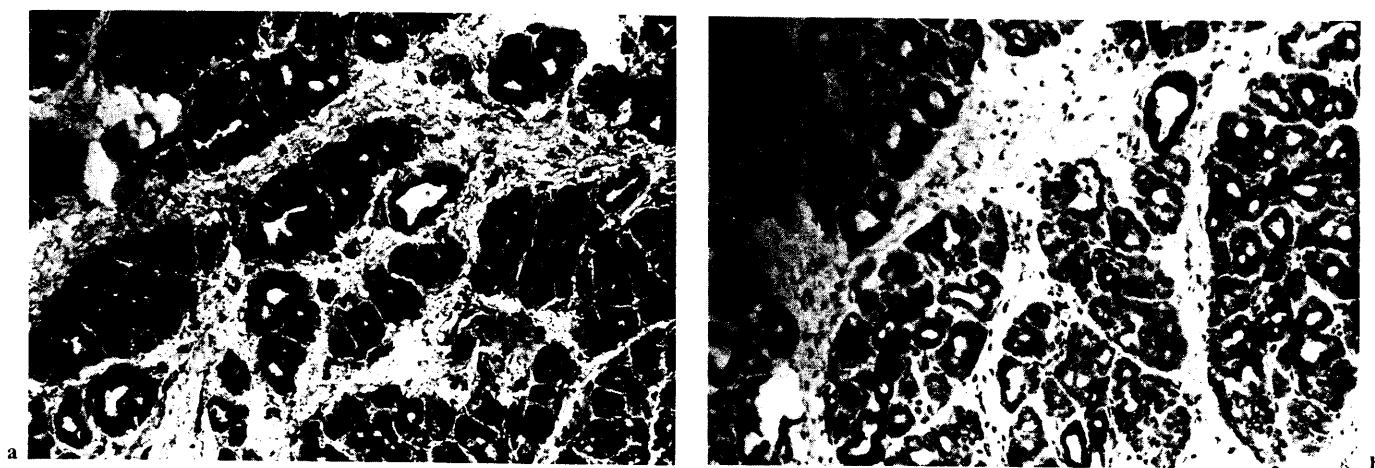


Fig. 2. **a** Tissue slice of the cat's lacrimal gland incubated with AF-labeled MAb CLB-Fd1a. Positive staining serous gland cells can be seen. 200 $\times$. **b** Negative control with MAb antiyellow jacket Ag 5.

cat dander extract from HAL (Haarlems Allergenen Lab., Haarlem, The Netherlands) was coupled (7.4 U Fel d I). This procedure was performed twice and resulted in a 3-fold reduction in anti-Fel d I activity.

As a control antibody we used protein A purified MAb to yellow jacket Ag 5 (1 mg/ml; anti-wasp MAb), also of the IgG1 isotype, instead of MAb CLB-Fd1a.

Results

Fel d I Assay

The concentrations of Fel d I in extracts of the cat's salivary and lacrimal glands and in the excretory products saliva, lacrimal fluid and milk (fig. 1) are listed in table 1.

Immunohistochemistry

Because of the low concentration of Fel d I in the submandibular gland, determined by a RIA, and the immunohistochemical studies of the sublingual gland done by Bartholomé et al. [8], we studied only the lacrimal and parotid glands. In tissue sections of the lacrimal gland the serous cells showed a strongly positive reaction to MAb CLB-Fd1a (fig. 2a); the serous cells of the parotid gland gave a weak staining. In both glands the ducts were negative.

In a control experiment the anti-Fel d I activity of MAb CLB-Fd1a was partially absorbed, which resulted in a 1:5 dilution. Compared to the untreated MAb, tested in the same dilution (0.2 mg/ml), the positive response of the lacrimal gland was distinctly reduced. The weak response of the parotid gland dis-

appeared after a 1:5 dilution of the untreated MAb, so no absorption experiments could be done. Incubation of tissue sections of the lacrimal and parotid glands with PBS or MAb anti-yellow jacket Ag 5 (fig. 2b), instead of MAb CLB-Fd1a, gave a negative result.

Discussion

In this study we investigated the presence of Fel d I in the cat's salivary and lacrimal glands. By means of a RIA, high concentrations of Fel d I were found in tissue extracts of the lacrimal gland, 500 mU/g tissue (= 1–2 μ g/g tissue). Immunohistochemical studies with MAb CLB-Fd1a confirmed this result; the serous cells of the lacrimal gland showed a strong specific reaction, which was abolished by preabsorbing the anti-Fel d I activity by solid-phase Fel d I.

The sublingual gland contained a similar amount of Fel d I, 610 mU/g tissue. This was in agreement with the study of Brown et al. [10] who found 480 mU/g tissue by quantitative immunoelectrophoresis and with immunohistochemical studies by Bartholomé et al. [8].

In our study Fel d I was also demonstrated in the parotid gland, at concentrations of 70–210 mU/g tissue, although in immunohistochemical studies its presence was difficult to demonstrate because of this low concentration. This presumably also explains why Bartholomé et al. [8] and Brown et al. [10] failed to detect Fel d I in the parotid gland (detection limit in the studies of Brown et al.: 240 mU/g tissue).

In the submandibular gland only very low levels of Fel d I were found, which is in accordance with the study of Brown et al. [10]. No relation was found between the type of gland and its Fel d I content. The glands containing the highest concentrations of Fel d I were the serous lacrimal gland and the partly serous (30%) partly mucous (60%) sublingual gland, both producing the enzyme lysozyme. Only low levels of Fel d I were found in the serous parotid gland and in the 80% serous, 5% mucous submandibular gland, which produces lysozyme [13]. Also, no correlation was found with amylase production.

The excretory products lacrimal fluid, saliva and milk were tested for their Fel d I content. The presence of Fel d I in excretes is presumably very relevant for allergic persons. The concentration of Fel d I in saliva was high, 4.2–7.0 U/ml, which is in agreement with the results of Brown et al. [10]. In lacrimal fluid a comparable concentration of Fel d I was found, 6.8–14 U/ml. The concentration of Fel d I in milk (0.33 U/ml) was low in comparison to saliva, but it was clearly present.

The results indicate that for cloning the Fel d I cDNA and for biosynthetic experiments the lacrimal gland appears to be the best source because of its high concentration of Fel d I and its larger total mass (70–80 mg) in comparison to the small sublingual gland (16 mg). The high concentration of Fel d I in lacrimal fluid can imply a high production of Fel d I, but we cannot rule out the possibility of lower secretion rates of lacrimal fluid.

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