

Cat skin as an important source of *Fel d I* allergen

A. J. Dabrowski, MD,* X. Van Der Brempt, MD,* M. Soler, MD,**
N. Seguret, MD,*** P. Lucciani, VD,**** D. Charpin, MD,* and
D. Vervloet, MD,* Marseille, France

It is generally recognized that cat skin is an important extrasalivary source of the major cat allergen, Fel d I. Nevertheless, since cat saliva is readily applied to the skin during grooming, we tried to demonstrate that Fel d I can accumulate on cat skin without licking. Eight adult female cats were used. On day 0, two separate sites of the trunk were shaved and a 6 cm² area in each site was washed three times with 5 ml of distilled water. The first and third washes were collected and stored for assay. The areas were then covered with an occlusive dressing. Twenty-four hours later, the site was washed once and the sample stored. On day 7, washes were collected as on day 0, and a collar was attached to the neck to prevent contamination from saliva. A single wash was done 24 hours later. Comparison of data was achieved with an analysis of variance. Repeated washes significantly reduced Fel d I levels from 35 ± 21 to 10 ± 9 mU/cm² on day 0 ($p < 0.01$) and from 36 ± 18 to 12 ± 7 mU/cm² on day 7 ($p < 0.01$). Fel d I levels increased significantly after 24 hours of occlusion from 10 ± 9 to 41 ± 24 mU/cm² ($p < 0.01$) and collar wearing from 12 ± 7 to 54 ± 21 mU/cm² ($p < 0.001$). These findings demonstrate that Fel d I is produced by cat skin. Mean daily production of Fel d I by cat skin is estimated to be 36 units. Cat skin is an important extrasalivary source of Fel d I. (J ALLERGY CLIN IMMUNOL 1990;86:462-5.)

Cat allergy is a frequent and well-documented cause of asthma and rhinitis.¹⁻³ The underlying mechanism involves specific IgE antibodies against antigen *Fel d I*, the major cat allergen.^{4, 5} In homes in which cats are kept, *Fel d I* is abundant in dust and air. Ohman et al.⁶ observed large quantities of *Fel d I* allergen in pelt washes from living cats. It has been postulated that *Fel d I* originates mainly from cat saliva and that the pelt is contaminated by licking during grooming.⁶⁻⁸ Since Bartholomé et al.⁹ demonstrated major cat allergen in hair roots and sebaceous glands of cat skin, it has been generally accepted that cat skin is an important extrasalivary source of this allergen.

Abbreviations used

Mab: Monoclonal antibody
PBS-LT: Phosphate-buffered saline-Tween

Nevertheless, it is essential to demonstrate that *Fel d I* can accumulate on cat skin without contamination. The work we report here is devoted to proving this finding.

MATERIAL AND METHODS

Study protocol (Fig. 1)

Eight healthy adult female domestic cats were individually caged and fed with a standard cat diet. On day 0, cats were anesthetized with ketamine HCl (15 mg/kg of weight, intramuscularly), and two separated areas of each trunk of the cats were carefully shaved. A 6 cm² area was outlined on the exposed skin and washed three times with 5 ml of distilled water through a plastic cylinder of the same diameter. Most of *Fel d I* amount present on cat skin was collected in the first (A) wash and was considered as the baseline antigenic level (Table I). Low level of *Fel d I* that could be collected in the third wash (B), was considered as the basis for comparison with the levels measured 24 hours later (Table I). This comparison allowed the demonstration and quantification of *Fel d I* antigen production by cat skin. The exposed areas were then occluded with plastic, gauze,

From the *Département des Maladies Respiratoires, Hôpital Sainte-Marguerite, **Laboratoire d'Immunologie, Hôpital Sainte-Marguerite, ***Clinique Vétérinaire (Dr. Cabassu), and ****Service Chirurgie-Animalerie, Laboratoire de Neurosciences Fonctionnelles, Centre National de la Recherche Scientifique, Marseille, France.

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Reprint requests: D. Vervloet, MD, Département des Maladies Respiratoires, Hôpital Sainte-Marguerite, BP 29, 13277 Marseille Cedex 9, France.

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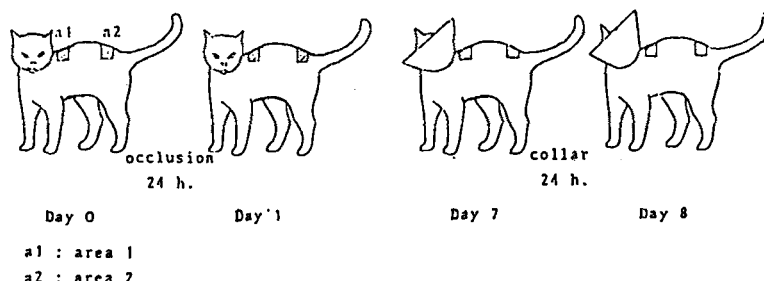


FIG. 1. Design of the study. Areas 1 and 2 were washed before and after 24-hour occlusion, and before and after 24-hour collar wearing.

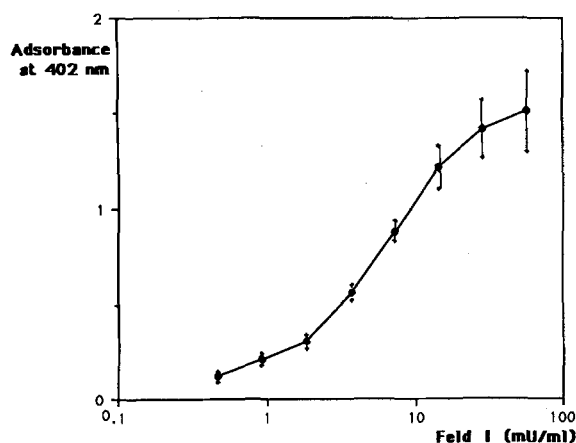


FIG. 2. Standard curve illustrating the correspondence between *Fel d I* concentrations and optical density.

and collodion to avoid contamination by saliva or surrounding fur. Twenty-four hours later on day 1, the areas were again washed one time. On day 7, washes were repeated after the same procedure as on day 0, and a collar was attached to the neck to prevent the cat from licking. The washes were repeated one time 24 hours later on day 8 as on day 1. All samples were stored until processing at -20°C .

Fel d I dosage

A two-site MAb-based ELISA was performed with *Fel d I*-specific MAbs provided by M. D. Chapman and TAE Platts-Mills (Charlottesville, Va.).¹⁰ Maxisorp F16, flat-bottom microtiter plates (Nunc Immuno module, Polyabo Paul Block et Cie, Strasbourg, France), were coated overnight with $1\text{ }\mu\text{g}$ per well of 6F9 MAb in bicarbonate buffer, pH 9.6, at 4°C . The plates were then washed twice with PBS-T and incubated for 30 minutes with 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.) or diluted samples were added to wells ($100\text{ }\mu\text{l}$ per well). Cat E3 is a cat-dander extract containing 10.5 U/ml with 1 unit equaling $5\text{ }\mu\text{g}$. Doubling dilutions of cat

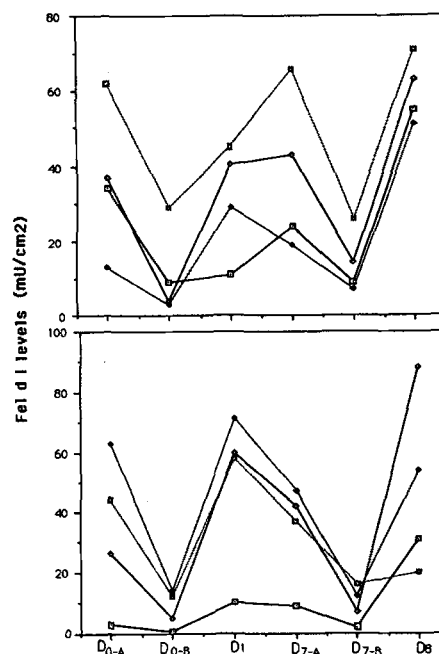


FIG. 3. Individual levels of *Fel d I* (expressed in milliunits per square centimeter of skin) measured in each cat during each experiment.

E3 were used to form a control curve from 58.3 to 0.5 mU/ml . Two dilutions of each sample (usually 1/1 and 1/4) were assayed twice. After a 1-hour incubation at room temperature, the plates were washed four times with PBS-T. Biotinylated 3E4 MAb was added ($100\text{ }\mu\text{l}$ per well of a 1/1000 PBS-T-bovine serum albumin dilution), and the plates were incubated for 1 hour and then washed four times with PBS-T. Streptavidin-peroxidase (Sigma Chimie Sarl, La Verpillière, France) was added ($100\text{ }\mu\text{l}$ per well of a 1/1000 PBS dilution or approximately 4×10^{-5} units per well), and plates were incubated for 30 minutes and washed four times with PBS-T. The substrate ortho-phenylenediamine (Abbott Laboratories, Rungis, France) was added (25.6 mg in 10 ml of solvent; $100\text{ }\mu\text{l}$ per well), and plates were incubated for 20 minutes in darkness. The reaction was stopped with $100\text{ }\mu\text{l}$ per well of H_2SO_4 2N.

TABLE I. *Fel d* I levels in skin washes expressed in milliunits per square centimeter

	Day					
	0-A	0-B	1	7-A	7-B	8
Cat area 1						
1	3.6	1.8	16.6	10.6	2.4	19.4
2	81.2	16.9	68.2	55.2	13.1	18.3
3	68.4	12.3	68.2	52.0	14.3	20.1
4	43.8	7.1	47.4	64.9	7.5	71.4
5	42.2	6.5	12.2	6.5	2.4	42.2
6	6.5	1.6	26.0	18.5	8.9	58.4
7	79.5	45.5	53.6	89.2	39.8	77.9
8	64.9	5.7	ND	71.4	24.4	68.2
Cat area 2						
1	3.2	0.4	3.4	6.5	1.6	43.0
2	45.0	11.0	73.0	39.0	10.0	89.0
3	19.0	11.0	47.0	21.0	17.0	20.0
4	8.0	2.0	73.0	20.0	6.0	104
5	25.0	11.0	10.0	41.0	15.0	67.0
6	19.0	4.0	32.0	20.0	6.0	44.0
7	45.0	12.0	37.0	42.0	12.0	65.0
8	9.0	3.0	ND	15.0	3.0	58.0
Mean ($\pm$ SD)						
Areas 1 and 2	35 $\pm$ 21	10 $\pm$ 9	41 $\pm$ 24	36 $\pm$ 18	12 $\pm$ 7	54 $\pm$ 21

A, First collection; B, third collection; ND, not done.

The optical density of each well was measured (Titertek Multiscan Plus MK II, Flow Laboratories, Eflaboy, Finland) and compared with values on the reference scale. Results were calculated as the mean of two wells at the same dilution and of the two dilutions of each sample. The lowest allergen concentration detected was about 0.5 mU/ml, that is, 2.5 ng/ml. The results were expressed in milliunits per square centimeter of cat skin. A typical standard curve with standard deviation is illustrated in Fig. 2. (Each point was done in triplicate.)

Statistical analysis. Comparison of the mean ($\pm$ SD) values was performed with an analysis of variance.

RESULTS

Occlusion was well tolerated in all but one case (cat 8, day 1), and the corresponding washes were excluded from analysis. In all, 94 cat-skin washes were assayed. All washes yielded measurable levels of *Fel d* I. Values ranged from 0.4 to 104 mU/cm² (Table I). The concordance between *Fel d* I levels in the two areas of each cat was fair. The mean of *Fel d* I levels measured on the two areas is presented in Table I. The basal *Fel d* I level (arithmetic mean $\pm$ SD) was comparable on day 0 (35 $\pm$ 21 mU/cm²) and day 7 (36 $\pm$ 18 mU/cm²). Repeated washes significantly reduced *Fel d* I levels from 35 $\pm$ 21 to 10 $\pm$ 9 mU/cm² on day 0 ($p < 0.01$)

and from 36 $\pm$ 18 to 12 $\pm$ 7 mU/cm² on day 7 ($p < 0.01$). *Fel d* I levels increased significantly after 24 hours of occlusion from 10 $\pm$ 9 to 41 $\pm$ 24 mU/cm² between day 0 and day 1 ($p < 0.01$) and collar wearing from 12 $\pm$ 7 to 54 $\pm$ 21 mU/cm² between day 7 and day 8 ($p < 0.001$) (Table I). Data points for each individual cat illustrate (Fig. 3) the innate variability of the data but also demonstrate a similar trend in all studied cats.

DISCUSSION

In our experiment, repeated lavages of cat skin readily diminished *Fel d* I levels on skin surface (Fig. 3). The fact that, following 24-hour intervals after washing, approximately the same quantity of *Fel d* I (in milliunits per square centimeter) can be collected demonstrates local production of *Fel d* I by cat skin. An occlusion device was used to prevent contamination of the skin by saliva or by antigens from surrounding fur. Since occlusion may stimulate the secretion by sebaceous glands, a second test was carried out with a collar to prevent licking. Thus, two different methods used to exclude cat saliva independently demonstrated the ability of cat skin to generate *Fel d* I in the absence of external application of cat saliva. Our results are in agreement with the histopathologic

work of Bartholomé et al.⁹ that suggested that sebaceous glands could be an important extrasalivary source of antigen *Fel d I*. Antigen *Fel d I* may be one of the proteins secreted by sebaceous glands and, since they are connected to hair roots, these glands may be involved in hair physiology. In their study of the distribution of allergen in cat tissues, Brown et al.⁷ observed the highest amount of *Fel d I* in pelt extracts. In this study, only female cats were used. This is important because regulation of skin sebaceous glands in cats has been demonstrated to be under hormonal rather than nervous control.¹¹ Androgens stimulate and estrogens inhibit secretory activity. Despite this finding, Brown et al.⁷ have found the highest concentration of *Fel d I* in female cat pelt. *Fel d I* levels on cat skin appear to be a balance between processes of production and elimination of the allergen.

Our data indicate that the daily production of *Fel d I* by cat skin is 36 mU/cm² (Table I). The skin surface of an adult cat can be roughly estimated by assimilating the animal to a cylinder 40 cm in length and 8 cm in diameter. Under these conditions skin surface is approximately 0.1 m². Based on this estimation, the daily production of an adult cat skin is 36 units of *Fel d I* (36 mU/cm² × 1000 cm²). Swanson et al.¹² recently reported that a single cat sheds about 5×10^{-4} units of *Fel d I* per minute (i.e., to 7.2 U/24 hr). At each step in our experiment, we observed some variability between cats and areas in the recovery rate of *Fel d I* from the skin. This variability could be linked to factors other than *Fel d I* production, namely, variations in the eluting technique, amount of natural oils on the skin, and amount of particulate *Fel d I* lost during the eluting process. In a recent study, allergen shedding was demonstrated to vary, not only between cats but also in the same cat, during the course of a day and between days.¹³

Cat fur appears to be a reservoir for the accumulation of *Fel d I* secreted by sebaceous glands and additionally from saliva. Additional studies will be

needed to quantify the relative importance of these two sources of *Fel d I* antigen.

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