

Effects of castration and testosterone on *Fel d I* production by sebaceous glands of male cats: I—immunological assessment

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Summary

Fel d I is produced by salivary and sebaceous glands. Hormonal control of sebum production is clearly established. The influence of cat castration and supplementary treatment with testosterone on the production of sebum and *Fel d I* in cat skin have been researched in this study. On day 1, 12 male cats were anaesthetized and three skin areas carefully shaven. Then the level of lipids on skin surface was measured by means of a photometric method. Finally, the three areas of skin were washed with 5 ml of distilled water through a plastic cylinder. *Fel d I* collected in the washes was measured with a two-site monoclonal antibody based ELISA. On day 2, six cats were castrated, the other six were used as a control group. Two and 4 weeks later, the levels of lipids and *Fel d I* in skin washes were measured again in all cats. On day 30, the six castrated cats were injected intramuscularly with prolonged-action testosterone. Two weeks later, quantification of lipids and *Fel d I* in all animals was repeated. Sebum and *Fel d I* levels decreased in all castrated animals. Injecting the castrated cats with testosterone led to a significant increase in sebum and *Fel d I* production. Our findings indicate that *Fel d I* production is influenced by the production of hormones.

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Introduction

Inhalation of animal proteins may cause an immediate-type allergic reaction in atopic persons [1–8]. Amongst domestic animals cats and dogs are the greatest source of allergy [5–8]. The major domestic cat allergen (*Fel d I*) is one of the most important animal antigens causing asthma and rhinitis in man [9–11]. This antigen is a glycoprotein produced by salivary glands and skin [12]. In contrast to general opinions, nowadays, skin is believed to be a producer of *Fel d I* independent of salivary glands [13,14]. Sebaceous glands produce this antigen in skin [15]. It is well known that sebum secretion by sebaceous glands is androgen-dependent [16–18]. In this study the production of *Fel d I* in skin after cat

castration and after supplementary treatment with testosterone was investigated.

Materials and methods

Twelve 1.5–2 year-old male European breed cats weighing between 2 and 2.5 kg were used for the study. Cats numbered 1–6 were kept as controls (group I) while cats numbered 7–12 made up the study group (group II). The animals were individually caged and kept at the same temperature (average of 21°C) and humidity (an average of 40%) conditions and fed with an identical standard commercial cat diet.

Study protocol

On day 0, cats were anaesthetized with ketamine HCl

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(15 mg/kg of body weight, intramuscularly). Three separate areas, 3 × 3 cm (A, B, C), of each cat were carefully shaven using an electric razor at exactly the same places in all cats (A: tail base, B: back of the neck, C: central part of abdomen). After cleaning areas A, B and C of cat skin, the level of lipids on their surface was measured by means of a photometric method. The areas (A, B and C) were then washed with 5 ml of distilled water through a plastic cylinder of 7 cm² according to the method described by Dabrowski *et al.* [14]. The wash samples were stored at -80°C. The level of *FeldI* antigen collected in washes was quantified with a two-site monoclonal antibody (MoAb) ELISA. On the following day, cats from group II were castrated under general anaesthesia. On day 14, cats were again anaesthetized, areas A, B and C were shaved and cleaned and quantification of lipids on skin surface and skin washes with the above methods were repeated; on day 28, quantification of lipids and skin wash of areas A, B and C in all cats was carried out for the third time. On the following day, group II was intramuscularly injected with hexahydrobenzoate testosterone 50 mg/kg. On day 42, assays of lipids and *FeldI* were repeated and the following day all castrated cats were again injected with the same dose of testosterone. The experiment was completed on day 56 with the succeeding, fifth quantification of lipids and skin washes of areas A, B and C in cats from both groups.

Lipids quantification

After cleaning the shaved areas A, B and C with gauze, the level of lipids was quantified with a photometric method (Lipometre® St L'Oreal licence) [19,20]. The technique evaluates sebum production since all the lipids collected on skin are of sebum origin. A special probe with a round glass plate (1.8 cm²) at its end was pressed on the exposed areas of cat skin for about 15 s and the degree of diminution of light transmitted across the round glass plate was computed. The light was emitted by a diode working at 650 nm. Transmitted light was detected and amplified by means of phototransistor. The transmission value of the fatted round glass plate was determined with respect to its zero value, that is, the initial transmission value obtained by using the clean round glass plate. The instrument was calibrated by the use of a standardization mixture of the lipidic components of the sebum. The result obtained was mathematically converted into µg/cm² of sebum. The reproducibility of the method was evaluated at around 5–8%. The final result was the arithmetical mean of measurements performed in four areas of the round glass

plate. After each quantification the round glass plate was cleaned with acetone to obtain the zero value of lipids on its surface.

FeldI dosage

FeldI dosage in the samples of skin was performed using a two-site MoAb-based ELISA with *FeldI*-specific MoAb provided by M. D. Chapman and T. A. E. Platts-Mills (Charlottesville, VA, USA) [21]. 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 0.05% Tween 20 (PBS-T), pH 7.4, 300 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 *FeldI* 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 13.3 to 0.5 mU/ml. Two dilutions of each sample (from 1/1 to 1/120) were assayed twice. After a 1-h incubation period at room temperature, the 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 the 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 reaction was stopped with 100 ml per well of H₂SO₄ 2N. The optical density of each well was measured at 492 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 0.5 mU/ml.

Statistical analysis

We computed sebum, as well as *FeldI*, differences between day 0 and day 28 on one hand, day 28 and day 56 on the other hand, in groups I and II, respectively. These differences were compared using an unpaired *t*-test. We then used an analysis of variance for repeated

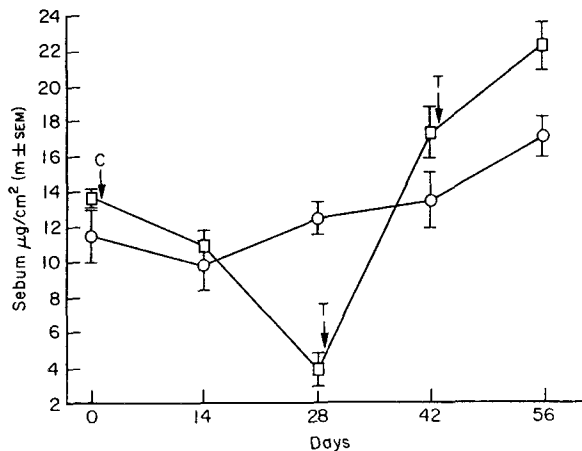


Fig. 1. Levels of sebum ($\mu\text{g}/\text{cm}^2$) in control group (group I $\circ$) and in group II: ($\square$) before, after castration and following injections of testosterone. Each symbol represents the mean value $\pm$ SEM calculated for three areas of each cat (18 values). C. Castration. T. Testosterone injection.

measurements and two controlled factors, i.e. group (control or treated) and event (castration, testosterone injection).

Results

Figure 1 presents the results of the variation in sebum concentration in groups I and II. Results are expressed as the mean value ($\pm$ the standard error of the mean SEM) in

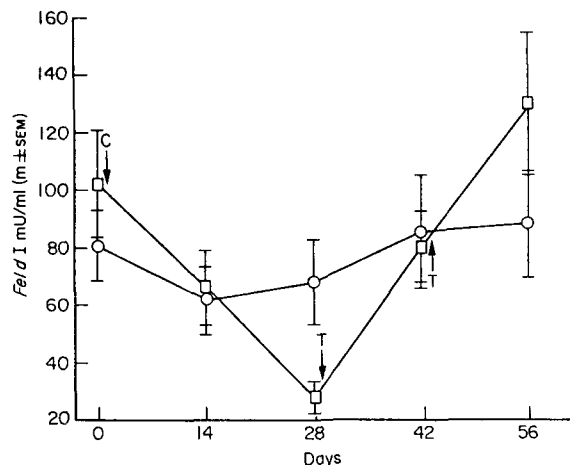


Fig. 2. Levels of *Fel d I* (mU/ml of skin washing) in control group (group I: $\circ$) and in group II ($\square$) before, after castration and following injections of testosterone. Each symbol represents the mean value $\pm$ SEM calculated for three areas of each cat (18 values). C. Castration. T. Testosterone injection.

Table 1. Analysis of variance with repeated sebum measurements using two controlled factors

Factor	d.f.	F	P
Group	1	0.2	0.7
Cat	10	5.0	0.0001
Event	4	228.5	0.0001
Group $\times$ event	4	96.4	0.0001

μg of sebum per cm^2 of skin. Each value is the mean of the results obtained for each cat in the three studied areas, A, B and C of the skin. No significant variations were observed in the control group. In contrast, in group II, a gradual decrease in sebum level was observed 1 week and 2 weeks after castration. Furthermore, after injections of testosterone, a gradual increase of the sebum levels was observed. The mean (SD) change in sebum concentration between day 0 and day 28 was equal to $1.6 \pm 5.4 \mu\text{g}/\text{cm}^2$ in group I, whereas it was equal to -12.7 ± 5.8 in group II ($P < 0.0001$). Between day 28 and day 56, the mean (SD) variations were equal to 4.5 ± 3.8 and 18.2 ± 5.1 in groups I and II, respectively ($P < 0.0001$).

Figure 2 gives similar results concerning variations in *Fel d I* levels. In the control group there was no statistically significant variation. In group II, as for the sebum concentration, there was a gradual decrease after castration. Later injection of the animals with testosterone, led to an increase in *Fel d I* levels. The mean (SD) change in *Fel d I* concentration between day 0 and day 28 was equal to $-12.3 \pm 27.2 \text{ mU}/\text{ml}$ in group I, where it was equal to -74.5 ± 59.1 in group II ($P < 0.003$). Between day 28 and day 56, mean (SD) variations were equal to 20.9 ± 34 and 103.0 ± 90.1 , in groups I and II, respectively ($P < 0.001$).

Tables 1 and 2 show the result of the analysis of variance with repeated measurements using two controlled factors, for sebum and *Fel d I* measurements, respectively. Among

Table 2. Analysis of variance with repeated *Fel d I* measurements using two controlled factors

Factor	d.f.	F	P
Group	1	0.2	0.9
Cat	10	23.7	0.0001
Event	4	11.2	0.0001
Group $\times$ event	4	4.6	0.004

the main factors, the groups (control or treated) did not show significant differences either for sebum or for *Fel d I* concentrations. In contrast, interaction between group and event was statistically highly significant ($P < 0.0001$ and $P = 0.004$, respectively) demonstrating that sebum and *Fel d I* levels show a very different trend following castration then testosterone injection (group II) than in the control group (group I).

Discussion

Antigen *Fel d I* has been found in saliva, extracts and washes of cats pelt [22–24]. It has been suspected to be produced mainly in salivary glands and to be passively transferred with saliva onto the pelt while licking [22,23]. The antigen has neither been found in serum nor in urine [23,24]. Bartholomé *et al.*, using a monospecific anti-serum and an immunofluorescence technique, demonstrated an extrasalivary source of *Fel d I* production in sebaceous glands [13]. Our previous works confirmed skin to be a *Fel d I* producer independent from salivary glands [14,15]. Using a monoclonal anti-*Fel d I* and an immunoperoxidase procedure, *Fel d I* has been reported to be present on the skin and pelt surface, in the basal cells of skin and in sebaceous glands [15]. Therefore, sebaceous glands seem to be the source of *Fel d I* in skin [13,15].

Sebaceous glands are holocrine, of epidermal origin, directly connected with the activity of hair roots, and produce sebum [16]. Sebum creates a protective lipid layer on the skin, giving slide to the fur and regulates the pH value of skin. Sebaceous glands may be recognized as secondary sexual organs because of direct dependence of sebum production upon androgens, mainly testosterone. Male cats produce more sebum than females [25]. Other hormones (oestrogens, progesterone, glucocorticosteroids or pituitary hormones) impede sebum production. Many other factors influence sebum production, i.e. age, sex, diet, drugs; nervous, genetic, physico-chemical and climatic factors (temperature and humidity) and irritants. It is difficult to determine the influence of a particular factor on the final effect, that is sebum production, which was ascertained in the present studies.

In the group of castrated cats a decrease in sebum level during 4 weeks after castration was observed. At the same time, in the control group the level remained stable. Injections of testosterone raised the production of sebum. These results demonstrate that sebum production is influenced by hormonal production. Since immunohistological evaluation revealed *Fel d I* antigen in sebaceous glands [13,15] the question arises as to whether sebaceous glands are the place of antigen production. The holocrine character of these glands

does not rule out this possibility. The finding that castration decreased the amount of *Fel d I* at the surface of the skin may indicate that, as for sebum, the *Fel d I* synthesis is influenced by hormones. However, another possibility cannot be totally excluded, i.e. that the sebum is the carrier of the *Fel d I* antigen permitting the passage of the antigen from the sebaceous glands to the surface of the skin. Reduction of the sebum would in this case decrease the passage of the *Fel d I* through the skin without affecting its production. Another hypothesis could be that androgens increase the synthesis of many proteins at large. However, the huge reduction in the presence of *Fel d I* on the skin surface 1 month after castration (decrease of more than 2/3) leads us to hypothesize a specific effect of androgen hormones on *Fel d I* production. However, since we did not take subsequent measurements after day 28, we do not know if the fall could be maintained over a prolonged period and therefore we cannot give any documented advice about castration of domestic cats in managing cat allergy. The role of *Fel d I* is not known but, similarly to uteroglobulin which has some similarity [12] *Fel d I* could play a role as a immunomodulating protein which can protect epithelium. Such a role would explain why this glycoprotein is produced by sebaceous glands which, because of the production of sebum create a protective lipid layer on the skin and regulate the pH value of the skin.

These results indicate that *Fel d I* production is influenced by hormones and could explain large between-cats and over-time variation in *Fel d I* shedding [26]. The results lead us to hypothesize that cat skin produces *Fel d I* in conjunction with sebum. Further work on the regulation of this protein, the possible differences between males and females, the biological activities of this major allergen, and the relationship between concentrations of major allergen and symptoms is needed.

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