

Fel d I allergen distribution in cat fur and skin

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Immunohistochemical procedures were performed to ascertain Fel d I antigen (Ag) distribution in cat fur and skin biopsy specimens and to analyze Fel d I allergen concentrations in fur. One hundred strands of fur and 24 skin biopsy specimens (6 by 4 by 3 mm) from shaved areas were collected from 11 different cats. Freshly depilated hairs were immunostained by free-floating monoclonal anti-Fel d I, avidin-biotin-peroxidase complex, and either processed for scanning electron microscopic examination or mounted on glass slides for computer-assisted densitometric analysis (SAMBA system). Skin biopsy specimens were promptly frozen and sectioned just before the immunohistochemical processing. Densitometric analysis of fur demonstrated that immunoprecipitate concentrations were tenfold higher at the root than at the tip. However, this finding may be explained by decrease of the thickness of the hair cortex that varied in similar proportions. The Ag accumulated on the strand surface but may focally penetrate into the medulla through the scale-like cortical interstices. In skin biopsy specimens, Fel d I Ag was found in epithelial squamous cells, within the epidermis and hair follicles, on the surface of the epidermis and hair follicles, and in sebaceous gland cells. These data suggest that Fel d I Ag is produced by sebaceous cells and, to a lesser extent, by basal squamous epithelial cells and that it is stored mainly on the surface of the epidermis and fur. (J ALLERGY CLIN IMMUNOL 1991;88:77-82.)

Key words: Fel d I allergen, immunoperoxidase, frozen sections, cat skin, cat fur, SAMBA analysis

According to studies published from different countries,¹ pets are kept in half or more households. Although dogs are more numerous, cat sensitization ranks first among pet allergies.² *Fel d I* is the major cat allergen. It was formerly considered to be produced by salivary glands and applied on cat fur by licking and grooming.³ However, in 1985, Bartholomé et al.⁴ suggested that *Fel d I* might originate from sebaceous glands. Recently, our group confirmed that cat skin is an important extrasalivary source of major cat allergen.⁵ In the present study, our goal was to investigate the fate of the allergen from sebaceous glands to roots and tips of the fur.

Abbreviations used

Ag: Antigen
Ab: Antibody

MATERIAL AND METHODS

Material

One hundred strands of fur were depilated from 11 different cats (six male and five female cats) with sterilized tweezers. All contact with the fingers was avoided to keep from rubbing the Ag off the fur and/or damaging the hair root. After fur strands were sampled, they were kept in Petri dishes at low temperature (+4° C).

Twenty-eight skin biopsy specimens, measuring 6 by 4 by 3 mm, were obtained from shaved skin areas to facilitate tissue sectioning, then embedded in a longitudinal position in the appropriate usual milieu (Miles Laboratories, West Haven, Conn.), promptly snap frozen before being immersed in liquid nitrogen, and stored at -80° C. Before immunostaining, each biopsy specimen was cut into five 5 µm sections (total, 140 sections) with an automatic cryostat (Frigocut 2800, Reichert-Jung GmbH, Nusslock, Germany). Sections were collected on coated glass slides (ER-ICA kit, Abbott Laboratories, Rungis, France).

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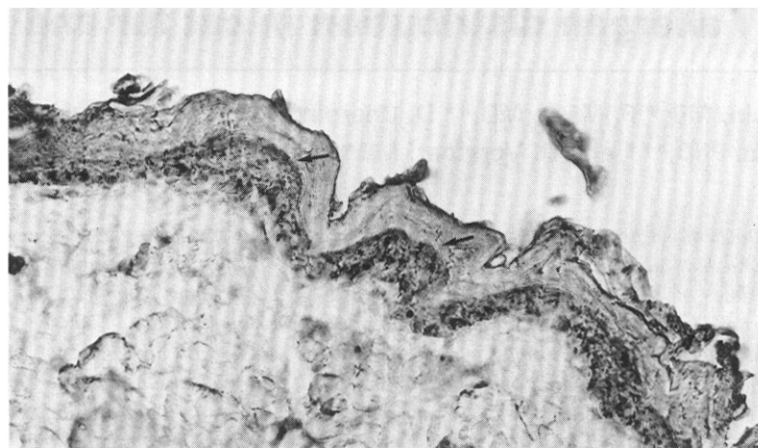


FIG. 1. Positive staining with anti-*Fel d I* Ab in basal cell of squamous cells (*arrows*) and on the surface of cat epidermis. (Original magnification $\times 120$.)



FIG. 2. *Fel d I* immunoreactive on the epidermis (*arrows*); the intensity of staining is stronger than in epithelial cells. (Original magnification $\times 300$.)

Rat, rabbit, monkey, and human skin specimens served as negative control biopsy specimens. Similarly, hair was sampled in rat, rabbit, and dog and used as negative controls.

Immunostaining procedures

Frozen skin sections and free-floating fur samples were immunostained with an immunoperoxidase procedure. *Fel d I* Ag was detected with a monoclonal anti-*Fel d I* supplied by M. Chapman and T. Platts-Mills. Specificity of the Ab was previously documented.⁶ The immunoperoxidase technique with an avidin-biotin-peroxidase complex and aminoethyl-carbazole has been described previously.⁷⁻⁹ Sections were counterstained with Mayer's hemalun. The usual negative controls⁷⁻⁹ of avidin-biotin-peroxidase procedure were performed (primary or second Ab omission) to evaluate the eventual background.

Microcytometry (SAMBA analysis)

The antigenic distribution was analyzed by detection of brown-reddish precipitates in a SAMBA computer-assisted

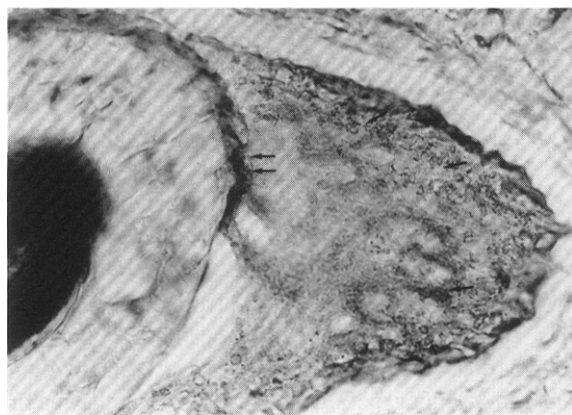


FIG. 3. Intradermal hair follicle (*transverse section*): positive *Fel d I* staining in squamous basal cells surrounding the hair in the follicle. Note increased antigen concentration (originating from both squamous and sebaceous cells) on the hair root surface (*arrows*). (Original magnification $\times 1200$.)

microcytometric system.^{10, 11} The program used was specifically designed to delete optical densities of the hair core. Data were expressed on a scale of arbitrary units ranging from 0 to 255. Densitometry parameters¹²⁻¹⁵ were measured in four different regions of each strand, that is, the root, the proximal external third, the intermediate external third, and the distal external third. Forty-five strands of various origins, that is, different cats and regions, were adequate for SAMBA analysis.

Scanning electron microscopy

Fresh unstained and immunostained hair was processed according to standard techniques for scanning electron microscopic examination (Jeol 35 CF SEM, Jeol France, Rueil-Malmaison, France).

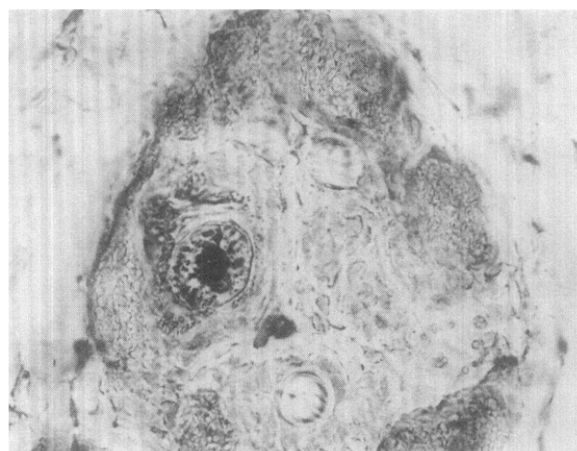
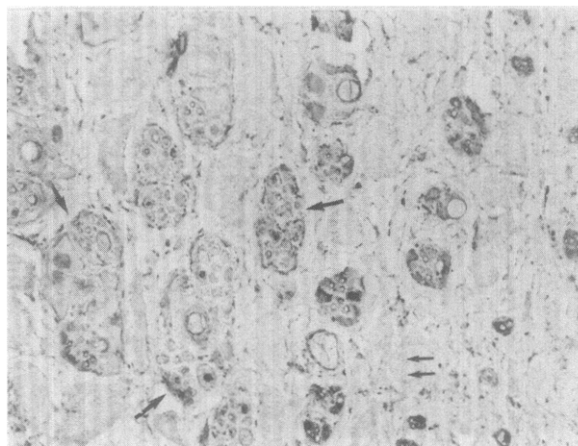


FIG. 4. Sebaceous gland immunostaining with anti-*Fel d* I. **Top,** Heterogeneity of the *Fel d* I distribution in sebaceous glands and cells. Positive glands (*single arrows*) are adjacent to unreactive glands (*double arrows*). (Original magnification $\times 120$.) **Bottom,** Positive *Fel d* I staining in sebaceous cells with vacuolated, lipid-rich cytoplasm (*arrows*). (Original magnification $\times 750$.)

RESULTS

Fel d I allergen distribution in tissue sections

In the immunostained tissue sections, *Fel d* I allergen was detected both intracellularly and extracellularly. Mild immunostaining was observed in squamous epithelial cells in the basal and intermediate layer in the form of intracytoplasmic clusters of various sizes (Fig. 1). Surprisingly, in contrast with the density of immunoprecipitates on the surface of the epidermis, no or only faint staining was observed on superficial epithelial cells (Fig. 2). Immunoreactive squamous cells were found on both the epidermis and squamous epithelium surrounding the fur root in the dermis (Fig. 3). In tissue sections in which the hair follicles were divided transversely, a target-like pattern resulting from the formation of pre-

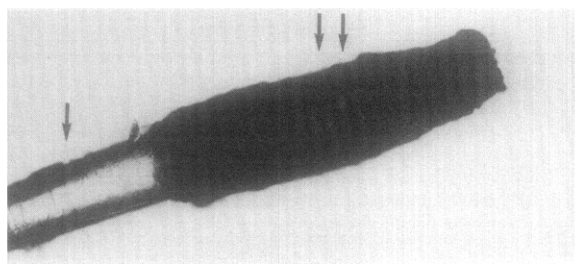


FIG. 5. *Fel d* I distribution in unsectioned strands of cat fur; strong immunoreactivity at root (*double arrows*) and in cortex (*arrow*). (Original magnification $\times 300$.)

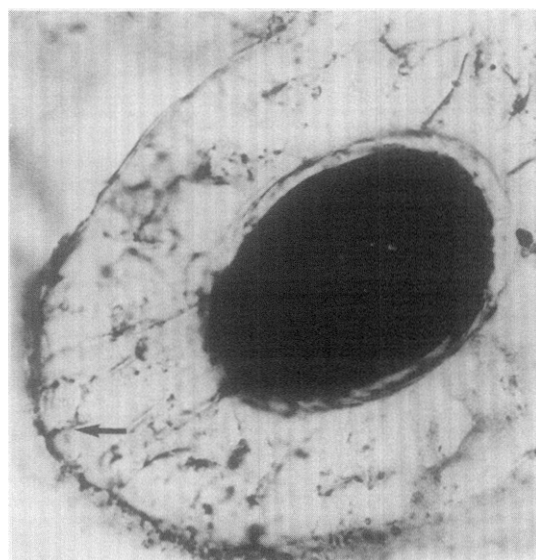


FIG. 6. Focal penetration of *Fel d* I Ag into the hair cortex through the scale-like structures. (Original magnification $\times 750$.)

cipitates around the hair core was observed (Fig. 3).

In the dermis, the sebaceous cells demonstrated various degrees of immunoreactivity. In most cases, diffuse immunostaining was observed in the vacuolated, lipid-rich cytoplasm of these large cells, measuring from 12 to 25 μm (Fig. 4). However, some sebaceous cells were partially or totally unstained (Fig. 4). The staining intensity was the same as on squamous cells but weaker than on the epidermis surface.

All biopsy specimens other than from cat skin specimens were *Fel d* I negative.

Fel d I distribution on hair

Only cat fur was *Fel d* I reactive. Negative controls were consistently unreactive (not presented).

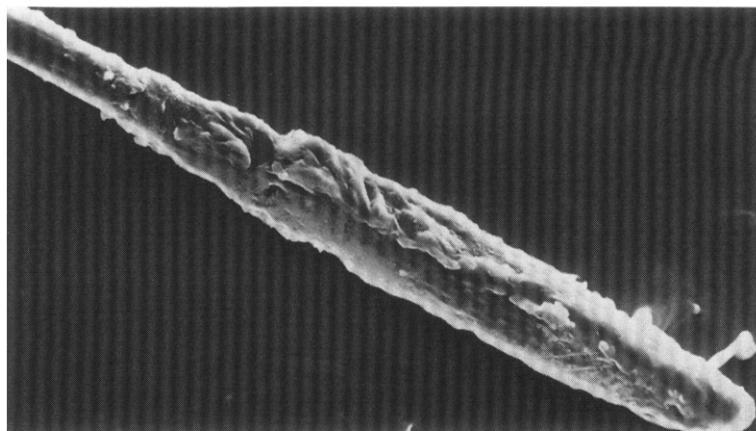


FIG. 7. Scanning electron microscopy illustrating hair root. Note the rough surface likely caused by lipidic and sebaceous aggregates containing the *Fel d* I antigen. (Original magnification $\times 450$.)

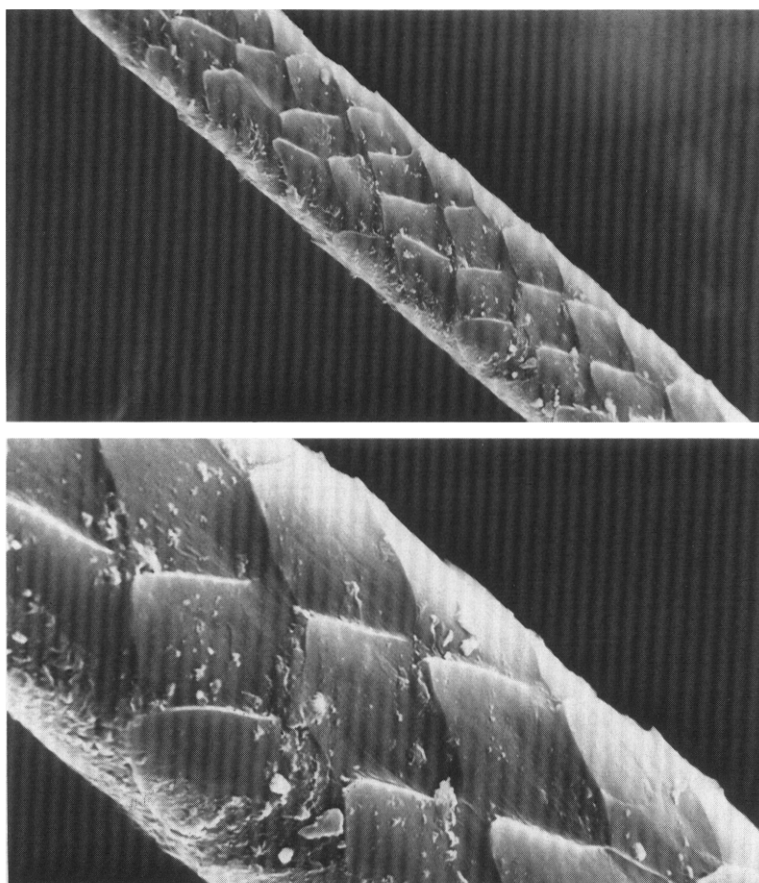


FIG. 8. Scanning electron microscopy illustrating the scale-like structures of the cat fur producing a "palm-tree" pattern. (Original magnification $\times 700$ and $\times 1700$.)

In unsectioned hair, the staining was noted over the whole surface and especially at the root. On the shaft, that is, the free portion of the strand, staining was located in the cortex (Fig. 5). Attempts to split the hair transversely after immunodetection failed because

the particular hardness of the core that could not be cut with standard blades. Because of artifacts, sections obtained with diamond knives were inappropriate for histologic evaluation. In some tissue sections, strands were transversely sectioned in the dermis. Examina-

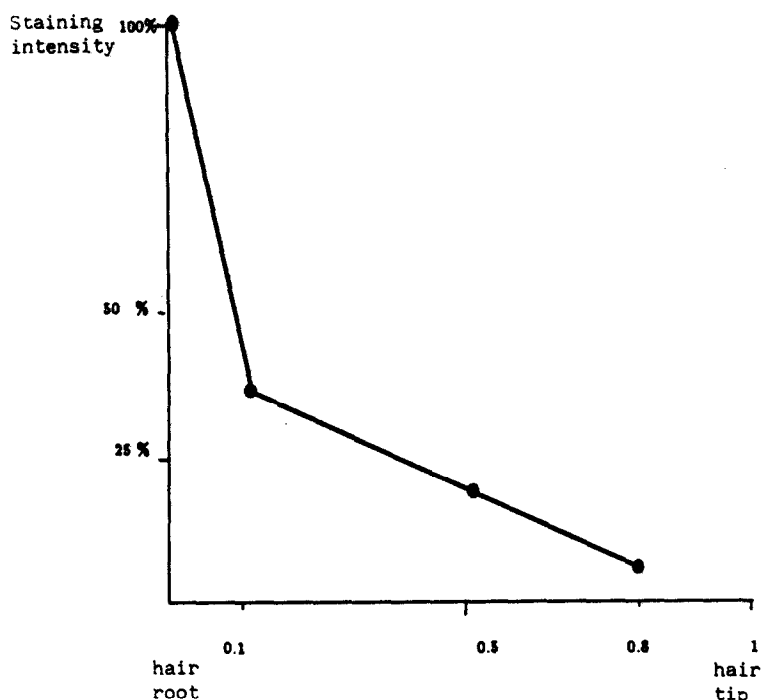


FIG. 9. SAMBA microcytophotometry. Optical densities of immunoprecipitates obtained with anti-*Fel d I* demonstrate a decrease of *Fel d I* from the root to the tip of the strand (abscissa: different hair levels; ordinate: mean values from measurements performed on 45 whole strands).

tion of these areas at high magnification demonstrated focal penetration of the reddish immunoprecipitates into the cortex along interstices between scale-like structures (Fig. 6). The hair medulla was unreactive.

Scanning electron microscopic study

Scanning electron microscopic study clearly revealed the scale-like features of the hair cortex and the rough surface of the hair root (Figs. 7 and 8).

Densitometric analysis of hair

SAMBA analysis demonstrated a decrease in the amount of immunoprecipitates from the root to the tip of the strand. The mean values of measurements performed in the four regions demonstrated that the Ag concentration at the root was tenfold higher than at the tip of the shaft (Fig. 9). These results strongly suggest that the *Fel d I* Ag found lying on the hair surface may originate, at least partly, from the hair follicle. However, it may simply reflect the analogous decrease in the thickness of the cortex from the root to tip.

DISCUSSION

In our study, *Fel d I* Ag, the major cat allergen, was detected in sebaceous glands and dermis cells. Bartholomé et al.⁴ reported similar findings. In our material, the distribution of *Fel d I* immunoreactive cells was heterogenous. Not all the cells were reactive,

and in individual cells, positive staining was variable. Moreover, some cells were completely negative and other cells strongly positive. There are three possible hypothesis to explain this heterogeneity. First, the antigenic epitopes detected by the monoclonal Ab used may not be accessible at all stages of *Fel d I* molecule development. Second, negative cells may engulf cells with a higher Ag metabolic rate. Third, negative cells are inactive and have no ability to store *Fel d I* in cytoplasm.

In frozen tissue sections, anti-*Fel d I* immunostaining was strong on the epithelial surface of the epidermis and hair follicle. This distribution contrasted with the weaker reactivity of the epithelial basal and intermediate cell layers and with little or no immunoreactivity on the epithelial superficial layer. These findings suggest that *Fel d I* Ag is essentially produced by basal squamous epithelial cells in the dermis and sebaceous glands and that after secretion it is spread from the root to the tip of the strand and over epidermis. Licking and grooming may enhance this spreading.

Scanning electron microscopy examination of cat fur revealed the particular scale aspect of the hair cortex. The orientation of these structures probably facilitates the spreading of the Ag from deep to superficial levels of the hair and its penetration into the cortex through the narrow interstices between scales. Scanning microscopic examination also dem-

onstrated the accumulate of particles along these scale-like structures but did not allow determination of their antigenic content. Transmission electron microscopic examination after preembedding immunostaining would be more suitable for this purpose. However, because of the hardness of the strands, attempts to section them produced changes in the Ag distribution and artifacts.

In conclusion, our findings strongly suggest that skin is a major source of *Fel d I* allergen found on cat hair and epidermis. In the skin, *Fel d I* appears to be produced mainly by sebaceous and basal squamous epithelial cells and stored on the surface of epidermis and hair. Ag produced in the intradermal hair follicle accumulates on hair roots and then is gradually spread to the tip of the shaft. From there it may become airborne and induce allergic respiratory symptoms.

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