

Evaluation of *Felis domesticus* allergen I as a possible autoallergen in cats with eosinophilic granuloma complex

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Objective—To investigate the role of *Felis domesticus* allergen I (Feld I) in the pathogenesis of eosinophilic granuloma complex (EGC) in cats.

Animals—7 healthy cats and 6 cats with EGC.

Procedure—Epidermis was removed from 4 areas. Rubber stoppers filled with Feld I, saline (0.9% NaCl) solution, and PBS solution were glued to the skin lesions and removed 48 hours later. Fluid within each stopper was collected. Biopsy specimens were obtained at each site, snap frozen, and stored at -70°C . Total and differential numbers of cells in fluid were counted. Biopsy specimens were stained by use of monoclonal antibodies against feline CD4, CD8 and CD3. Data were analyzed by use of multivariate repeated-measures analysis.

Results—Healthy cats had a significant increase in number of CD3+ cells, compared with number of CD4+ and CD8+ cells, and Feld I caused a significant increase in number of CD3+ cells, compared with PBS or saline solutions. Cats with EGC had a significant increase in number of CD3+ cells, compared with number of CD4+ and CD8+ cells, and Feld I caused a significant increase in number of CD3+ and CD4+ cells, compared with PBS or saline solutions. Cats with EGC had an increased CD4+ response, a significantly decreased CD8+ response, and a significantly increased CD4-to-CD8 ratio compared with healthy cats.

Conclusions and Clinical Relevance—The increased CD4+ response, significantly decreased CD8+ response, and significantly increased CD4-to-CD8 ratio are comparable to results in atopic people and allergic cats. Therefore, Feld I could be an autoallergen responsible for chronic inflammatory reactions in cats with EGC. (*Am J Vet Res* 2002;63:338–341)

Cats are an important source of allergens for humans.¹ Sensitization to cat allergens is an important cause of asthma or rhinitis in humans. In 1 study,² 20 to 30% of asthmatic patients responded to prick tests that involved the use of cat allergens. A large part of the IgE antibodies in patients allergic to cats is directed against the cat allergen *Felis domesticus*

allergen I (Feld I).^{3–5} Important sources of Feld I are cat saliva and hair, in which Feld I is probably the result of production by the skin and sebaceous glands and by deposition of the allergen during grooming.^{6,7} The production of Feld I is under hormonal control and higher in male than female cats.⁸

The Feld I molecule is relatively stable. Only a modest reduction in Feld I activity is found after heating. It is a heterodimer with a molecular weight of 35 to 39 kd, consisting of 2 chains of 17 to 18 kd.¹ Cloning and sequence analysis of Feld I revealed that the mRNA coding for these 2 chains are the products of separate genes that are expressed in the salivary glands and skin of cats.^{9,10} The biological role of Feld I in cats remains unknown.⁷

Atopic dermatitis (AD) in humans is a chronic inflammatory skin disease that often affects individuals with a genetic predisposition to increased IgE synthesis and IgE-mediated hypersensitivity reactions evident in the form of allergic rhinitis and asthma.¹¹ Transepidermally absorbed aeroallergens can crosslink IgE at the surface of dermal mast cells, and aeroallergens can activate mononuclear cells via IgE bound to IgE receptors on Langerhans cells.^{12,13} In humans with atopy, IgE recognizes exogenous allergens as well as structurally related human proteins (ie, so-called autoallergens). These results suggest that there is IgE autoimmunity in patients with AD and that it may be of pathogenic relevance for the chronic nature of dermatologic manifestations in this disease.^{14–19} The immunopathogenesis of AD in cats has strong similarities to that of AD in humans.^{20–23}

The eosinophilic granuloma complex (EGC) in cats includes a group of 3 lesions: an indolent ulcer, an eosinophilic granuloma, and an eosinophilic plaque.²⁴ In the past, this complex was considered to be a separate disease entity. Currently, this complex is considered to be a reaction pattern of the skin and mucosa. Cats with EGC are often observed licking the area associated with erosive or ulcerative skin or mucosal lesions. Although the clinical signs have been described in detail, the underlying pathogenesis is still unclear. In some cases, hypersensitivity reactions such as atopy or food hypersensitivity have been suggested as predisposing factors,²⁴ but in many cases, an underlying cause cannot be detected. The role of Feld I in these reactions is unclear.

The study reported here was conducted to test the hypothesis that Feld I is absorbed through cutaneous or mucosal barriers as the result of licking or other microtrauma in cats with EGC, thereby inducing an allergic reaction. To test this hypothesis, we used a skin blister technique²⁵ and immunohisto-

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chemical analysis to describe the effects of Feld I on the skin in healthy cats and cats with clinical signs of EGC.

Materials and Methods

Animals—Two groups of cats were used in the study. One group consisted of 7 healthy (control) cats (5 females and 2 castrated males) that were between 7 and 8 years old (median, 7 years). The second group consisted of 6 cats (3 castrated females and 3 castrated males) with EGC that were between 1 and 12 years old (median, 4 years). Four of these cats had an indolent ulcer on the upper lip, 1 cat had an indolent ulcer on the upper lip and an eosinophilic granuloma on the tongue, and 1 cat had an eosinophilic plaque on the left hind limb. The diagnosis of EGC was made on the basis of clinical and histopathologic features.¹ Except for the described skin and mucosal problems, these cats also were healthy. None of the cats received injections of glucocorticoids for at least 6 weeks or received orally administered antihistamines or glucocorticoids for at least 4 weeks prior to the study.

Blister technique—In both groups of cats, a suction technique was used to create blisters, as described elsewhere.²⁵ Briefly, each cat was anesthetized (medetomidine, 0.1 mg/kg, IM), and the hair on the lateral aspect of the thorax was clipped. A plexiglass vacuum block with 4 polished holes was placed on the clipped skin. A vacuum of -20 cm Hg was created and maintained until blister formation was observed. The epidermis over each blister was removed. Sterile rubber stoppers^a were filled with Feld I (2 stoppers), saline (0.9% NaCl) solution (1 stopper), and sterile PBS solution (1 stopper) and glued to the skin lesions. The Feld I was used at a concentration of 18 µg/ml, which is comparable to the concentration in cat saliva.⁶ Affinity-purified Feld I^b was used in the study.²⁶

Forty-eight hours later, the stoppers were removed, and fluid within each was collected. Immediately after removal of the stoppers, a 6-mm biopsy specimen was obtained from each of the sites of the skin lesions. Skin specimens were immediately snap frozen in liquid nitrogen and stored at -70 C until analyzed.

Total number of cells in fluid obtained from each stopper was counted by use of an automated counter.^c Smears of fluid were stained by use of May-Grünwald Giemsa, and differential cell counts were performed.

Immunohistochemical analysis—Immunohistochemical analysis was performed for samples obtained from both groups of cats, as described elsewhere.²² Briefly, biopsy specimens (6 µm) were air-dried overnight and fixed in acetone for 7 minutes. Nonspecific binding was blocked by prior incubation for 25 minutes with PBS solution containing 10% horse serum and 10% cat serum. Primary antibodies were murine monoclonal antibodies against feline CD4, CD8,²⁶ and CD3.²⁷ Control stain was an isotype-matched antibody. Positive-staining cells in the superficial dermis were counted by use of a square reticule in 4 adjacent HPF (400X magnification). Number of cells in 4 sequential sections of each skin biopsy specimen was counted, and values were added. Cells in hair follicles and deep dermis were excluded.

Statistical analysis—Multivariate repeated-measures analysis was used for the immunohistochemical data to compare the number of stained cells after provocation of skin with Feld I, PBS solution, and saline solution in healthy cats and cats with EGC. This analysis also was used to compare values between these groups of cats. Values of $P < 0.05$ were considered significant.

Results

Total and differential cell counts—In healthy cats, total cell counts in fluid varied between 21×10^9 and 27×10^9 cells/L, consisting of 94 to 98% neutrophils and 2 to 6% lymphocytes. In cats with EGC, total cell counts varied between 14×10^9 and 48×10^9 cells/L, consisting of 91 to 99% neutrophils and 1 to 9% lymphocytes. Eosinophils were not found in either group. Cell counts did not differ significantly between the 2 groups.

Immunohistochemical analysis—Microscopic examination in both groups revealed that the epidermis was almost completely removed by the suction blister procedure. We did not observe serious damage in the dermis. Sections stained with isotype-matched antibody did not reveal positive staining. Infiltrates of T cells were most prominent in the superficial dermis in both groups. The cell infiltrate was sometimes focal with a large number of positively stained cells seen in 1 HPF but no positively stained cells in an adjacent field.

In healthy cats, there was a significant increase in number of CD3+ cells, compared with number of CD4+ and CD8+ cells (Table 1). Use of Feld I caused a significant increase in number of CD3+ cells, compared with number of CD3+ cells for PBS solution and saline solution. The CD4-to-CD8 ratio for Feld I, PBS solution, and saline solution was 0.76, 0.59, and 0.60, respectively.

In cats with EGC, number of CD3+ cells also was significantly increased, compared with number of CD4+ and CD8+ cells (Table 2). Use of Feld I caused a significant increase in number of CD3+ and CD4+ cells, compared with number of CD3+ and CD4+ cells for PBS solution and saline solution. The CD4-to-CD8 ratio for Feld I, PBS solution, and saline solution was 2.06, 2.11, and 1.26, respectively.

Comparing values for the healthy cats with

Table 1—Mean ± SEM number of positively stained cells in skin biopsy specimens obtained from 7 healthy cats 48 hours after blisters were induced by use of a suction technique and the skin was exposed to various compounds

Compound	CD3	CD4	CD8
Feld I	16.1 ± 1.7 ^{A,a}	5.8 ± 0.8 ^b	7.6 ± 0.8 ^b
PBS solution	9.7 ± 2.3 ^{B,a}	3.7 ± 0.4 ^b	6.2 ± 0.9 ^b
Saline solution*	10.0 ± 2.2 ^{B,a}	5.0 ± 0.9 ^b	8.2 ± 1.3 ^b

*0.9% NaCl solution.
 Feld I = *Felis domesticus* allergen I.
^{A,B}Within a column, values with different superscript letters are significantly different ($P < 0.05$). ^{a,b}Within a row, values with different superscript letters are significantly different ($P < 0.05$).

Table 2—Mean ± SEM number of positively stained cells in skin biopsy specimens obtained from 6 cats with eosinophilic granuloma complex 48 hours after blisters were induced by use of a suction technique and the skin was exposed to various compounds

Compound	CD3	CD4	CD8
Feld I	12.5 ± 1.3 ^{A,a}	9.9 ± 0.9 ^{Ab}	4.8 ± 1.0 ^b
PBS solution	9.2 ± 2.8 ^{B,a}	5.7 ± 0.7 ^{Bb}	2.7 ± 0.6 ^b
Saline solution*	9.9 ± 2.5 ^{B,a}	5.3 ± 1.0 ^{Bb}	4.2 ± 1.4 ^b

See Table 1 for key.

those of the cats with EGC, there was an increased, but not significantly different, number of CD4+ cells in the cats with EGC. In cats with EGC, there was a significant decrease in CD8+ response and a significant ($P = 0.006$) increase in the CD4-to-CD8 ratio, compared with values for healthy cats. We did not detect other significant differences between the 2 groups.

Discussion

Eosinophilic granuloma complex in cats comprises 3 clinical syndromes (ie, an indolent ulcer, an eosinophilic plaque, and a linear granuloma). The underlying cause in all of these syndromes is unknown, although they may be considered to be reaction patterns of hypersensitivity.²⁴

In contrast to humans, little is known about the pathogenesis of AD in felids. In cats with AD, there is a perivascular-to-diffuse infiltrate of mast cells, eosinophils, lymphocytes, and macrophages, comparable to the infiltrate observed in humans with AD.²⁸ Additionally, there is a familial involvement in cats with AD.²⁴ Reaginic (IgE) hypersensitivity in cats has been documented by use of passive cutaneous anaphylaxis testing in cats infested with *Otodectes cynotis*.²⁰ In addition, cats with miliary dermatitis or eosinophilic plaques have evidence for the existence of a heat-stable cytophilic antibody.²¹ Only recently has a feline epsilon heavy chain with substantial sequence homology to those of other species been cloned.²⁹ Roosje et al^{22,d} reported a predominate increase of CD4+ T cells in lesional skin of allergic cats and proved that feline CD4+ T cells in lesional skin produce interleukin 4, supporting a role for Th2-dependent pathways in the pathogenesis of the disease. Additionally, it was found that Langerhans cells, related dermal dendritic cells, and other major histocompatibility class-II+ cells may actively participate in AD in cats.²³ On the basis of those results, there is abundant evidence that the pathogenesis of AD in cats and humans has strong similarities.

When comparing values for healthy cats with those of cats with EGC, we found that cats with EGC had a nonsignificant increase of CD4+ cells and a significant decrease of CD8+ cells, which was reflected in a significant increase of the CD4-to-CD8 ratio. These findings are analogous to results for humans with AD³⁰ and results described in lesional skin of cats with AD²²; therefore, it was extremely suggestive of an allergic reaction to Feld I in cats with EGC. To explain this allergic reaction, we developed the following hypothetical sequence: cats lick themselves frequently as part of normal behavior. Cats may have stress or behavior problems that result in intense licking. Intense licking with the rough surface of their tongue may damage the epidermis and result in Feld I penetrating the skin. Further licking may induce IgE-mediated allergic inflammatory reactions against Feld I in some cats.

Another mechanism could be that intense licking in pruritic cats with an existing allergic dermatitis boosts the inflammatory reactions as a result of the allergic reaction against Feld I. In our study reported here, intense licking of the skin was imitated by the suction blister technique. With this technique, a major

portion of the epidermis was removed. Contact between skin and Feld I was imitated by use of the rubber stoppers filled with Feld I.

In our hypothesis, autosensitization is considered to be an important pathogenic factor for the chronic inflammation in EGC. This situation may be comparable to AD in humans in which a substantial proportion of individuals mount IgE responses to allergens of mammalian origin and even to human epithelial cell-derived proteins (ie, autoallergens).¹⁴⁻¹⁹ To elucidate the exact role of Feld I, serum of cats with EGC should be tested by use of IgE against Feld I and by use of IgE autoreactivity against cat proteins. To document the antigen-specificity of the increased T-cell response to Feld I in future experiments, a nonspecific protein should be tested as an extra control treatment in the suction blister technique. Additional investigations also are required to elucidate the role of T-cell subsets in the pathogenesis of EGC in cats.

^aRubber stoppers, IGT, Zeewolde, The Netherlands.

^bProvided by the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands.

^cMicrocell counter, Goffin Meyvis, Etten Leur, The Netherlands.

^dRoosje PJ, Van Kooten PJS, Thepen T, et al. A role of Th2 cells in the pathogenesis of allergic dermatitis in cats?. Joint Congress of the British and Netherlands Society for Immunology (abstr). *Immunology* 1995;86(suppl 1):98-W3.3.

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