Evaluation of skin test reactivity to environmental allergens in healthy cats and cats with atopic dermatitis

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Objective—To evaluate skin test reactivity to environmental allergens in healthy cats and in cats with atopic dermatitis (AD).

Animals—10 healthy cats and 10 cats with AD.

Procedure—10 allergens in serial dilutions were injected ID on the lateral aspect of the thorax of sedated cats. Histamine (0.01% solution) and buffer solutions were used as positive and negative controls, respectively. Immediately after the last injection, 10% fluorescein solution was administered IV. Skin test results were evaluated with ultraviolet light after 15 to 30 minutes and at 4 and 6 hours by 2 independent observers. In the control group, skin tests were repeated after 6 weeks. Skin test reactivity and the nature of the immunoglobulin involved were investigated by use of the Prausnitz-Küstner test with untreated and heat-treated cat sera.

Results—Intertest and interobserver agreement were high when measurement of the diameter of the fluorescent wheal was used to evaluate skin test responses, compared with assessment of its intensity. In both groups of cats, immediate skin test reactivity was observed as an IgE-mediated reaction, as an IgG-mediated reaction, and as a result of nonspecific mast cell degranulation. There was no correlation between allergen concentration and the type of reaction observed.

Conclusions and Clinical Relevance—Skin test reactivity in cats should be evaluated after IV administration of 10% fluorescein solution by means of a Prausnitz-Küstner test to differentiate among IgE-mediated, IgG-mediated, and nonspecific reactions. (*Am J Vet Res* 2003;64:773–778)

Since the early 1980s, **atopic dermatitis (AD)** has become recognized increasingly as an important cause of pruritic skin disease in cats.[1,2] Concomitant respiratory problems are occasionally seen with AD.[3] A prevalence of AD in ≤ 73% of all allergic cats has been reported.[2,4] Pruritic miliary dermatitis, head and neck pruritus and dermatitis, and eosinophilic plaques are the most important clinical manifestations.[5] Atopic dermatitis in cats is usually diagnosed on the basis of history of pruritus, the presence of crusted papules, response to glucocorticoid treatment, histologic characteristics of affected skin, skin test results, and the exclusion of other cutaneous diseases, such as food hypersensitivity, dermatophytosis, parasitic skin diseases, and fleabite hypersensitivity.[6,7] In cats with AD, histologic examination of skin samples reveals perivascular and interstitial dermal infiltrates that consist of mast cells, eosinophils, lymphocytes, and macrophages,[8,9] which are similar cellular components to those observed in AD of humans.[10] The results of intradermal tests led initially to the hypothesis that atopy in cats is also mediated by reaginic antibodies.[5,11-14] In cats infested with *Otodectes cynotis*, IgE hypersensitivity has been revealed by means of passive cutaneous anaphylaxis testing.[15] Baldwin et al[16] and Foster et al[17] found evidence for a putative feline IgE. Cross-reactivity between monoclonal anticanine IgE and a putative feline IgE in sera of cats experimentally parasitized with *Toxocara canis* has been observed,[12] and specific polyclonal antifeline IgE antibodies have been found.[18] Moreover, McCall et al[19] identified feline IgE with an FcεR1α-based assay. In addition to IgE, a heat-stable cytophilic antibody was found in cats with miliary dermatitis or eosinophilic plaques; the nature of this antibody and its pathogenetic importance are still unknown.[20]

In a study[21] of affected skin of allergic cats, Roosje et al found high concentration of $CD4^+$ T cells, which is similar to the finding of studies[22,23] in humans. Participation of antigen-presenting Langerhans cells in the pathogenesis of AD in cats has also been confirmed,[24] and there is evidence for the enhanced proliferation of Th2-like subsets.[25]

Although the immunopathogenesis of AD in cats is becoming increasingly understood, causative allergens have mainly been identified via positive intradermal immediate skin test reactions.[2,5,26-28] Difficulties associated with these tests are known and include poor and inconsistent wheal formation, inability to assess skin test results because of the rapid disappearance of the injected allergen solution, use of inadequate (nonstandardized) concentrations of allergen test solutions, and the influence of stress.[6,18,28,29] Therefore, the purpose of the study reported here was to evaluate skin test reactivity to environmental allergens in healthy cats and cats with AD by means of fluorescein sodium administered IV and the **Prausnitz-Küstner (P-K)** test.

Materials and Methods

Animals—Twenty cats were included in the study. Ten of the cats were clinically healthy European shorthair cats that belonged to the Utrecht University Animal Hospital. Nine of those cats (5 spayed females and 4 neutered males; age range, 7.1 to 9.2 years [median, 7.4 years]) were included in the control group, and one 7-year-old neutered male was used exclusively for the P-K tests. Ten client-owned cats

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were included in the study after referral to the Utrecht University Animal Hospital for evaluation of AD. In this group, there were 7 European shorthair cats, 1 Burmese, 1 Abyssinian, and 1 Abyssinian crossbreed; the cats were 2.1 to 15.8 years of age (median, 5.3 years). Four of the 10 cats were neutered males and 6 were neutered females. All 10 cats had clinical signs compatible with AD[6] (miliary dermatitis [n = 6], head and neck pruritus [5], eosinophilic plaques [2], or a combination thereof). Physical examination revealed no other abnormalities. Ectoparasitic infestation and dermatophytosis were ruled out by dermatologic examination, microscopic examination of skin scrapings and hairs, Wood's light examination, fungal culture of plucked hairs, and history of administration of flea control products for at least 2 months prior to evaluation. None of the cats had any clinical improvement after a 6-week period on a home-prepared elimination diet. Results of histologic examination of multiple skin biopsy specimens obtained from each cat were compatible with the diagnosis of AD.[6,9,10]

Intradermal allergy tests—Each cat was sedated with medetomidine (100 µg/kg, IM), and the hair of an unaffected area of skin on the lateral aspect of the thorax was gently clipped. An IV catheter was placed in a cephalic vein. Intradermal allergy testing was performed according to standard procedures.[6] Four serial dilutions of 10 allergens were tested. *Dermatophagoides pteronyssinus* and *D farinae* were tested at solution concentrations of 50, 100, 200, and 400 **Noon units** (**NU**)/mL, and solutions of grass pollen mixture, perennial rye grass (*Lolium perenne*), Kentucky bluegrass (*Poa pratensis*), Bermuda grass (*Cynodon dactylon*), birch (*Betula* spp), mugwort (*Artemisia vulgaris*), lamb's quarter (*Chenopodium album*), and cat flea (*Ctenocephalides felis felis*) were tested at concentrations of 500, 1,000, 2,000, and 4,000 NU/mL. Tests included PPS-solution as the negative control sample and 0.01% histamine solution as the positive control sample.[a] For each test, 0.05 mL of each allergen solution was injected ID. Immediately after injection of the test solutions, 10% fluorescein sodium solution (4.4 mg/kg) was injected via the IV catheter to facilitate assessment of the skin test response. The skin was examined under ultraviolet light in a darkened room at 15 to 30 minutes and 4 and 6 hours after injection of the allergens. With few exceptions, 2 observers independently evaluated each test, unless indicated otherwise. The intensity of the fluorescence was classified subjectively in a semiquantitative manner, such that a score of 0 = very weak, 1 = weak, 2 = medium, 3 = intensive, and 4 = very intensive. The long axis (mm) of the fluorescent wheal was recorded. Reactions were considered positive when the long axis of the wheal was equal to or larger than half of the sum of the axes of the respones to the 2 control solutions.[30] In cats of the control group, each test was carried out twice (test 1 and 2) with an interval of ≥ 6 weeks to determine the intertest variation. In cats with AD and the healthy cat used for the P-K tests, the intradermal allergy test was carried out only once.

Prausnitz-Küstner test—Blood samples were obtained from all cats at the time of the allergy test (in the control group; test 1) by venipuncture of the jugular vein; 2 mL of serum was separated from each sample and stored at –20°C until used in the P-K test. Briefly, a healthy cat (without skin test reactivity) that was used exclusively for the P-K tests was sedated with medetomidine (100 µg/kg, IM); 0.1-mL aliquots of serum from each cat with skin test reactivity were injected ID. The number of injected aliquots in this cat was equal to the number of positive skin test reactions in cats in the AD and control groups. After 24 hours, the cat was sedated, and 0.05 mL of the allergen solution at the concentration that had resulted in a positive skin test reaction initially was injected ID at each serum injection site. Phosphate-buffered saline solution was injected ID as a negative control. Immediately after performing these injections, 10% fluorescein sodium solution (4.4 mg/kg) was injected IV. Skin test reactions were interpreted at 15 to 30 minutes after injection of the allergens. A reaction was considered positive if the diameter of the fluorescent wheal was equal to the diameter of the wheal induced by the negative control solution plus ≥ 5 mm.[12,31] A serum sample that induced a positive reaction in this P-K test was subsequently maintained at 56°C for 2 hours to inactivate IgE antibodies.[32] The P-K test was then repeated on the same cat with this inactivated serum sample.

Statistical analyses—For cats of the control group and cats with AD, skin test results were recorded for each allergen in all concentrations by both observers. The results of all skin tests, including measurement of diameter and intensity of fluorescence of the wheals as well as the reproducibility between the 2 consecutive tests in control cats and between the 2 observers, were analysed by use of **kappa** (**κ**) statistics. With this method, the chance-expected agreement, which depends on the prevalence of the various diagnostic categories, is removed from the assessment of agreement, which is then defined as the κ value.[33,34] The calculations were performed with computer software.[b]

Table 1—Results of intradermal allergy tests for *Dermatophagoides farinae*, grass mixture, mugwort, and cat flea (*Ctenocephalides felis felis*), expressed as proportion of positive reactions (number positive/number of cats tested and evaluated by each observer) in healthy cats

Allergen	Concentration (noon units/mL)	Test 1		Test 2	
		Observer 1	Observer 2	Observer 1	Observer 2
D farinae	50	0\9	0\6	0\9	0\3
	100	0\9	0\6	0\9	0\3
	200	0\9	0\6	0\9	0\3
	400	1\9	1\6	0\9	0\3
Grass mixture	500	0\9	0\6	0\9	0\3
	1,000	0\9	0\6	0\9	0\3
	2,000	0\9	0\6	0\9	0\3
	4,000	0\9	0\6	1\9	0\3
Mugwort	500	0\9	0\6	0\9	0\3
	1,000	0\9	0\6	0\9	0\3
	2,000	0\9	0\6	1\9	0\3
	4,000	0\9	0\6	0\9	0\3
Cat flea	500	1\9	1\6	1\9	0\3
	1,000	0\9	0\6	2\9	1\3
	2,000	2\9	2\6	2\9	1\3
	4,000	2\9	2\6	2\9	1\3

Table 2—Agreement (Kappa [κ] value) between test 1 and test 2 and observer 1 and observer 2, with regard to the long axis of the fluorescent wheals and intensity of fluorescence in 9 healthy cats evaluated by use of fluorescent intradermal tests with various allergens

Variable	Comparison	Measure of agreement (κ)	Asymptotic SE
Long axis of the fluorescent wheal (mm)	Test 1 vs test 2	0.804* 0.825†	0.78
	Observer 1 vs observer 2	1.000‡ 1.000§	0.00
Intensity of fluorescence of the wheal	Test 1 vs test 2	0.174* INS†	0.34
	Observer 1 vs observer 2	0.775‡ 0.802§	0.03 0.05

*Observer 1. †Observer 2. ‡Test 1. §Test 2.
INS = Insufficient data.

Results

Intradermal allergy tests—In the cats of the control group, no reactions were seen in skin tests 1 or 2 performed with *D pteronyssinus*, perennial rye grass, Kentucky bluegrass, Bermuda grass, birch, and lamb's quarter. Immediate skin test reactivity was observed to certain concentrations of *D farinae*, grass mix, and mugwort in only 1 cat each; in 2 cats, immediate skin test reactivity to cat flea allergen was observed at all concentrations (**Table 1**). The grass mix and mugwort reactions detected in test 2 developed in 1 cat that was evaluated by observer 1 only. No skin test reactions were observed 4 and 6 hours after allergen injection.

Agreement between test 1 and test 2 was high (κ = 0.804 and 0.825, respectively; **Table 2**) when evaluation of the skin tests was based on measurement of the long axis of the fluorescent wheals (a reaction was considered positive when the wheal axis was equal to or larger than half of the sum of the axes of wheals associated with the control solutions). Measure of

Table 3—Results of the intradermal allergy test and Prausnitz-Küstner (PK) tests with unheated and heated serum of cats with AD

Allergen	Concentration (X1,000 NU/mL)	Cat No. (clinical signs)*				
		2 (EP)	3 (MD)	4 (HNP, MD)	6 (HNP, EP)	9 (MD)
D farinae	0.05		+	0		
	0.1		+			
	0.2	0	0	0	0	
	0.4		+	0	+	
D pteronyssinus	0.05			0		
	0.2			0		
Grass mix	0.5			0		
	1	0		0		
	2			0		
	4		0			
Perennial rye grass	1			+		0
	2		0	0		0
	4			0		
Kentucky bluegrass	0.5			0		
	1			+		
	2			0		+
	4			0		+
Bermuda grass	0.5			0		
	1			0		
	2			0		
	4			0		
Birch	0.5			0		0
	1		0	0		
	2		+	0		
Mugwort	0.5			0		
	1			0		
	2		+	+		
	4		+	0		
Lamb's quarter	0.5			0		
	1		0			
Cat flea	0.5		0	+		
	1			+	#	
	2			+		
	4	+		+	+	

MD = Miliary dermatitis. EP = Eosinophilic plaques. HNP = Head and neck pruritus and dermatitis. + = Positive results of intradermal allergy test, positive results of P-K test (unheated serum), and negative results of P-K test (heated serum). # = Positive results of intradermal allergy test, positive results of P-K test (unheated serum), and positive results of P-K test (heated serum). 0 = Positive results of intradermal allergy test and negative results of P-K test.
*Cats number 1 (MD), 5 (HNP), 7 (MD), 8 (HNP, MD), and 10 (HNP) have not been included in the table because they had no skin test reactivity.

agreement between the 2 observers was 100% ($\kappa = 1.00$ in both tests) when the evaluation of the skin tests was based on measurement of the long axis of the fluorescent wheals. In contrast, for observer 1, results of evaluation of the skin tests by assessment of the intensity of fluorescence of the wheals on a semiquantitative scale (0 to 4) had poor agreement between test 1 and test 2. For observer 2, there were insufficient data to calculate a κ value for this parameter. However, agreement between the 2 observers was high ($\kappa = 0.775$ [test 1] and 0.802 [test 2]). Hence, only the fluorescent wheal axis was used to evaluate skin test reactivity in the P-K testing of the control cats' serum and all further tests in cats with AD.

In cats with AD, positive skin test results were detected with *D farinae* (4/10 cats), cat flea (4/10), birch (3/10), mugwort (2/10), Kentucky bluegrass (2/10), perennial rye grass (3/10), grass mixture (3/10), Bermuda grass (1/10), *D pteronyssinus* (1/10), and lamb's quarter (2/10). Five cats did not have a positive skin test reaction to any of the allergen dilutions (**Table 3**).

P-K tests—The reactions to cat flea in cat No. 1 of the control group could be transferred with both unheated and heated serum. With the exception of the reactions to grass mix (4,000 NU/mL), all reactions in cat No. 6 of the control group could be transferred with unheated serum; reactivity was eliminated after heating.

The P-K test with unheated serum was performed with sera from the 5 cats with AD that had exhibited a positive skin test result initially. Nineteen of 55 (34%) test sites yielded positive results in the P-K test (Table 3). There was no correlation between the number of nontransferable positive skin test results and the concentration of the allergen. Exposure of the serum to heat (56°C for 2 hours) eliminated all positive P-K test results, with the exception of that associated with 1 cat with AD (No. 6) to cat flea allergen at a concentration of 1,000 NU/mL. Interestingly, the positive test result to the same antigen at a concentration of 4,000 NU/mL was eliminated. In all cats, no adverse reactions to the IV administration of fluorescein or the allergy tests were seen.

Discussion

All cats in the AD group met the criteria that are proposed for the diagnosis of AD in cats,[2,5,7,35] whereas none of the cats in the control group had any signs of pruritus or skin disease.

To the authors' knowledge, only Bevier[11] has evaluated serial dilutions of allergens in healthy cats or cats with AD; with the exception of house dust, some pollens, mixed feathers, and whole body-flea antigen, that investigator recommended use of the same allergen concentration as applied in dogs (1,000 protein nitrogen units (PNU)/mL). However, slightly different criteria for the evaluation of the tests were applied; turgidity and color were assessed in addition to diameter of the wheals. Positive skin test reactions were not further defined by means of P-K or **passive cutaneous anaphylaxis (PCA)** tests. Hence, the nature of these reactions (immunoglobulin [IgE or IgG]-mediated or not) remains unclear. In our study, no association between allergen solution concentration and development of response or type of skin test reactivity could be established. Thus, we concluded that the tested allergens did not act as irritants. On the basis of measurement of wheal axis, the intertest and interobserver agreement was high, whereas evaluation of the intensity of skin test reactions after IV administration of 10% fluorescein sodium solution yielded poor agreement. Our data suggested that measurement of wheal diameter is the best means with which to evaluate skin test reactivity. This variable can be accurately determined by the use of the fluorescein technique. In our study, no adverse reactions to IV administration of fluorescein were noted. Davidson et al[36] reported an apparent anaphylactic reaction associated with IV administration of 10% fluorescein sodium solution in a cat; however, the dose of fluorescein in that cat was approximately 5 times that used in our study, and there was no conclusive proof of anaphylaxis. Therefore, in our opinion, there is insufficient indication of risk associated with the administration of fluorescein, as used in our study. In our study, cats received medetomidine IM at a dose of 100 µg/kg. No data are available about the effects of medetomidine on skin test reactivity in cats, because cats have traditionally been anesthetized with ketamine,[6] ketamine-diazepam,[6] or tiletamine-zolazepam.[37] In dogs, medetomidine is considered not to affect skin test reactivity.[30] The appearance of the histamine-induced wheal in all cats of our study may indicate that medetomidine does not negatively affect wheal formation.

The importance of skin test reactivity in an individual animal is dependent on the animal's clinical status and the type of the test reaction. Additionally, skin test reactivity is an indication that an animal has been sensitized but does not necessarily indicate clinical allergy. In both groups of cats in this study, 3 types of positive skin test results were seen. Some positive skin test reactions were transferred to control cats via unheated serum in the P-K test, but this transference was not reproducible after exposure of the serum to heat. Such reactions are IgE-mediated, because IgE is a thermolabile immunoglobulin and becomes denaturated with heat.[32,38] In the P-K test, transference of positive skin test results via unheated and heated serum indicated that the reactions were IgG-mediated or IgE- and IgG-mediated because of the thermostability of IgG.[32] Other positive skin test reactions were not transferred via unheated or heated serum in the P-K test. These reactions are perhaps not the result of IgE- or IgG-mediated (nonspecific) mast cell degranulation, or they are not mast cell mediated. Skin test reactions are not always associated with allergen-specific immunoglobulin in serum,[17] and this may explain in part the nontransferable skin test reactions in our study. In this study, reactions induced by mast cell degranulation in response to the injection itself occur more frequently in cats with AD, compared with control cats. The higher density of mast cells in AD cats may be an explanation[39]; alternatively, the threshold for mast cell degranulation may be lower in cats with AD. In such circumstances, consistent mast cell degranula-

tion with all allergen concentrations above this threshold would be expected. In our study, these reactions occurred at random.

Passive transfer of immediate hypersensitivity-like responses in mice sensitized against trinitrophenol by ID injection of immunoglobulin-free light chains that are specific for trinitrophenol has been reported.[40] Results of that study also indicate that increased plasma extravasation can be induced in PCA tests by purified specific light chains alone and that this response is independent of host immunoglobulin and is mediated by mast cell degranulation. Immunoglobulin-free light chains have also been described in humans and rabbits; they are produced and secreted by plasma cells in greater amounts than immunoglobulin-heavy chains.[41,42] It is unknown whether this phenomenon also occurs in cats. Because of the skin test reactions observed in our study, the role of immunoglobulin-free light chains in mast cell degranulation in intradermal allergy tests, as well as in the pathogenesis of AD, is a matter of speculation. At this time, a P-K or PCA test is the test of choice to identify these reactions.

Only 1 cat (No. 1) of the control group and 1 cat with AD had an IgG-mediated reaction against flea antigen. Although cats are considered to be the primary host of *C felis*, little is known about the immunologic response to flea antigen in cats with and without flea allergy dermatitis. In dogs, IgE and IgG antibodies against different flea antigens have been reported.[43] It is likely that the immune response to flea antigen is similar in both species.

IgE-mediated reactions to various allergens were identified in 1 cat (No. 6) of the control group and in 5 cats with AD. In this control cat, IgE-mediated reactions were observed in only 1 of the 2 tests. Theoretically, sensitization during the test interval may have developed, but the first test may have been conducted improperly. The 5 cats with AD met the criteria for clinical diagnosis of AD, and therefore an IgE-mediated allergy was likely. Five other cats had negative results of the skin test despite clinical and histologic evidence of AD. This was probably attributable to the limited allergen panel, which was restricted to 10 allergens.

The type of skin test reactivity (IgE-mediated, IgG-mediated, and nonspecific reactions) varied among concentrations of the same allergen. Mast cell heterogeneity, as reported in other species,[44,45] and competition between IgE, IgG, and immunoglobulin-free light chains for sites on the mast cell surface offer possible explanations for these results.

In our opinion, the fluorescein technique greatly improves the evaluation of intradermal allergy tests in cats, but wheal reactions should be evaluated on the basis of measurement of the long axis of the fluorescent wheal and not the intensity of the fluorescence. Moreover, it is advisable to test allergens at multiple dilutions, and skin test reactivity should be evaluated by means of P-K tests with heated and unheated serum to identify the type of reactions.

[a]ARTU Biologicals Europe, Lelystad, The Netherlands.
[b]SPSS for Windows 10.1, SPSS Inc, Chicago, Ill.

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