

Immunophenotyping of the cutaneous cellular infiltrate after atopy patch testing in cats with atopic dermatitis

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Abstract

Cats with spontaneously occurring atopic dermatitis have clinical and immunocytochemical characteristics compatible with these in humans with atopic dermatitis (AD). The atopy patch test (APT) has proven to be a valuable tool in elucidating the disease process in humans. Additionally, the APT is very specific and bypasses the problem of conflicting results due to differences in chronicity of lesions of AD patients.

We adapted the APT for use in cats to explore the suitability of the APT as a tool to study the onset of allergic inflammation in cats with atopic dermatitis.

APT were performed in AD cats ($n = 6$) and healthy cats ($n = 10$). All cats were patch tested with two allergens in three different dilutions and a diluent control. The allergens for the APT were selected from positive intradermal test and/or prick test results and consisted of: *Dermatophagoides farinae*, *D. pteronyssinus*, *Tyrophagus putrescentiae*, and a grass pollen mixture. APT were read after 10, 24 and 48 h, and punch biopsies for immunohistochemical evaluation were collected at these time points.

Macroscopically positive APT reactions were observed in three out of six cats at 24 and/or 48 h with allergen concentrations of 25 000 and 100 000 NU/ml. Reactions were not observed at negative control sites and neither in control animals.

A significantly increased number of IL-4⁺, CD4⁺, CD3⁺, MHC class II⁺ and CD1a⁺ cells was found in one AD cat with positive APT reactions. Five out of six AD cats had significantly increased IL-4⁺ T cell numbers at 24 and/or 48 h.

Our data indicate that in cats, macroscopically positive patch test reactions can be induced, which have a cellular infiltrate similar to that in lesional skin. We found a high specificity and a macroscopically positive APT reaction in half of the cats, which is similar to what is seen in humans. Hence, the APT in cats might be a useful tool in studying the immunopathogenesis of feline atopic dermatitis.

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Keywords: Allergen; Atopic dermatitis; Atopy patch test; Cats; T cells

Abbreviations: APT, atopy patch test; AD, atopic dermatitis; Dfar, *Dermatophagoides farinae*; Dpt, *Dermatophagoides pteronyssinus*; Tyro, *Tyrophagus putrescentiae*; GP, grass pollen; DC, diluent control

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1. Introduction

Atopic dermatitis (AD) in humans is in most patients associated with increased allergen-specific IgE and is a disorder with a multifactorial pathogenesis including genetic, environmental, immunologic and pharmacologic factors (Leung, 1999). In addition, a relationship with allergen exposure and state of disease was demonstrated (Adinoff et al., 1988; Ring et al., 1991). A double-blind controlled trial, testing the effect of house dust mite allergen avoidance in atopic dermatitis, confirmed this relationship (Tan et al., 1996).

The epicutaneous application of aeroallergens, the atopy patch test (APT), has been widely used and has proven to be a good model for studying allergic inflammation in the skin of humans (Clark and Adinoff, 1989; Darsow et al., 1996; Langeveld-Wildschut et al., 1996; Seidenari et al., 1992). The APT bypasses the problem of conflicting results due to differences in chronicity of the lesions of AD patients. Analysis of the cytokine pattern expressed in situ revealed a biphasic pattern with an increased expression of IL-4 mRNA and IL-2 mRNA at 24 h and an increased expression of IFN- γ mRNA and IL-2 mRNA at 48 h compared to control skin (Grewe et al., 1995). These findings were confirmed at the protein level by (Thepen et al., 1996), who described a predominance of IL-4⁺ T cells over IFN- γ ⁺ T cells in 24 h APT reactions and an increased number of IFN- γ -producing cells compared to IL-4-producing cells in 48- and 72-h APT reactions, and in chronic lesional skin. A comparable switch in cytokine production has been observed in acute versus chronic AD skin. In acute lesional AD skin, more IL-4 mRNA-expressing cells were found than in chronic lesional skin (Hamid et al., 1994).

Various observations in cats with AD indicate similarity to the human disease. Feline atopic dermatitis is a commonly occurring pruritic, recurrent skin disorder that has a young age onset (Scott et al., 2001). Familial occurrence suggests a role for genetic factors (Cieslicki and Cieslicki, 1989; Moriello, 2001; Scott and Miller, 1993). The diagnosis is dependent on clinical signs, compatible histopathology and exclusion of other disease such as parasitic disease, dermatophytosis, flea bite hypersensitivity and food hypersensitivity reactions (Scott et al., 2001). Positive

intradermal test reactions towards common aeroallergens are often observed (Scott et al., 2001).

At present, however, limited information is available on the pathogenesis in cats. The histopathology of feline AD is characterised by a perivascular to diffuse dermal infiltrate with lymphocytes, mast cells, eosinophils, and macrophages (Gross et al., 1992). Several studies indicate the existence of a putative feline IgE (Baldwin et al., 1993; Foster et al., 1995; Gilbert and Halliwell, 1998; Griot-Wenk et al., 2000; Schleifer and Willemse, 2003; Weber et al., 2000). We described an increased number of CD4⁺ T cells compared to CD8⁺ T cells in lesional skin of allergic cats (Roosje et al., 1998) and demonstrated an increased IL-4 expression by CD4⁺ T cells in lesional and non-lesional skin of AD cats (Roosje et al., 2002). Significantly increased numbers of CD1a⁺ cells and MHC class II⁺ cells were found in the epidermis and dermis of AD cats, suggesting a pathogenic role of Langerhans cells and possible cutaneous penetration and presentation of allergens (Roosje et al., 1997).

The aim of this study was to investigate if epicutaneously applied allergens can induce macroscopically positive APT reactions in AD cats and to characterise the cellular infiltrate at the APT skin sites of cats with spontaneous AD and healthy cats by immunophenotyping.

2. Methods

2.1. Animals

Two castrated male and three spayed female, domestic, short-hair cats, and one castrated male, Norwegian Forest cat with ages between 1.5 and 11 years (median 5 years), with chronic and recurrent glucocorticoid-responsive pruritus and dermatitis, a histopathology of lesional skin indicating allergic inflammation (Gross et al., 1992), and positive reactions to intradermal testing were included in this study. Parasitic skin diseases, dermatophytosis and food hypersensitivity were ruled out by standard diagnostic procedures (Scott et al., 2001). Except for the skin problems, the animals were healthy. All AD cats were withdrawn from glucocorticoids for at least 6 weeks and received no antihistamines or oral glucocorticoids for a minimum of 4 weeks prior to skin testing.

Five castrated male cats and five spayed female, healthy, domestic, short-hair cats were used as control animals. Their ages ranged between 1 and 7 years (median 4 years). APT and prick test were applied to the control animals in the way same as that in the AD cats.

The healthy control cats were kept under conditions as demanded by the University of Utrecht. The study was approved by the committee for use of experimental animals, University of Utrecht.

2.2. Allergy skin tests

In addition to the intradermal test (ARTU Biologicals Europe B.V., Lelystad, the Netherlands), which is the standard method for skin testing in cats and dogs (Scott et al., 2001), a prick test was performed at the same time. In the prick test, a selection of seven allergens was used that was also included in the intradermal skin test (*Dermatophagoides farinae*, *D. pteronyssinus*, *Tyrophagus putrescentiae*, grass pollen mixture (*Dactylus glomerata*, *Phleum pratense*, *Syn-dactylon*, *Anthax odoratum*, and *Holcus lanatus*), *Poa pratensis*, *Lolium perenne*, and *Ambrosia eliator*). Of each allergen/allergen mixture (ARTU Biologicals Europe B.V., Lelystad, the Netherlands), three concentrations were used (5000, 25 000, and 100.000 NU[Noon Units]/ml) together with a positive control (1% histamine solution) and a negative control solution (allergen diluent solution). The allergen diluent solution consisted of a buffered sterile solution of 0.37% sodium chloride, 0.40% phenol, sodium dihydrogen phosphordihydrate, and disodium hydrogen phosphordihydrate (pH 7.30–7.50) (buffer solution, ARTU Biologicals Europe B.V., Lelystad, the Netherlands).

Both allergy tests were performed while the cats were sedated with 10 mg/kg ketamine, 1 mg/kg xylazine, and 50 mcg/kg atropine sulphate, administered intramuscularly. In the prick test, drops of the allergen dilutions and control solution were applied on the clipped, non-lesional, lateral side of the thorax with the cat in lateral recumbency. The skin was pierced through the drops with a lancet (Unilet GP superlite, Owen Mumford Ltd., Oxford, England). The skin was observed for 30 min after pricking and reactions were interpreted at 15-min interval after pricking with

transillumination and palpation of the prick site. Reactions were considered positive when a distinct erythematous papule was visible and palpable in absence of a reaction of the negative control dilution. The prick test was performed in control cats as in the AD cats.

2.3. Atopy patch test (APT)

The patch test in AD cats and healthy control animals was performed according to the procedure described for humans by (Langeveld-Wildschut et al., 1995). On each animal, three patch strips containing seven discs each (Finn chambers, Epitest Ltd. Oy, Tuusula, Finland), one for each time point of evaluation: 10, 24, and 48 h, were applied on non-lesional skin of the thorax at 24 h after clipping and chemical epilation of the hairs (Opilca cream, Schwarzkopf & Henkel, Düsseldorf, Germany), and after 10× skin stripping with adhesive tape (Leukofix, Beiersdorf AG, Hamburg, Germany). The strips were protected with an elastic bandage. The allergens (ARTU Biologicals Europe B.V., Lelystad, the Netherlands) were applied on the discs in the three standard dilutions (5000, 25 000, and 100 000 NU/ml). Because of the limited feline body size, only two allergens in three dilutions and a diluent control could be tested. The allergens for the APT were selected from positive intradermal test and/or prick test results and consisted of: *D. farinae*, *D. pteronyssinus*, *Tyrophagus putrescentiae*, and a grass pollen mixture. In the control group, five cats were tested with *D. farinae* and *D. pteronyssinus* and five other cats were tested with *T. putrescentiae* and grass pollen mixture dilutions and a diluent control for each time point.

2.4. Biopsies

Four-millimetre punch biopsies were taken under sedation with medetomidine HCl (100 µg/kg i.m.) from the macroscopically positive patch test sites and the diluent control sites at 10, 24, and 48 h after application. When there were no positive reactions visible, the site of the highest concentration was biopsied. After biopsy collection, cats received atipamezole HCl (250 µg/kg i.m.) to antagonise the effect of medetomidine. Skin biopsies

were immediately snap-frozen in liquid nitrogen, and stored at -70°C until used.

2.5. Immunohistochemistry

Sequential biopsy sections ($6\text{ }\mu\text{m}$) were air-dried overnight and fixed with acetone for 7 min. Non-specific binding was blocked by preincubation for 25 min with phosphate-buffered saline (PBS) containing either 10% horse serum and 10% cat serum, or 10% goat serum and 10% cat serum, depending on the secondary antibodies used. Primary antibodies were murine monoclonal antibodies against feline CD1a (FE 1.5F4) (Woo and Moore, 1997), MHC class II (42.3) (Rideout et al., 1992), CD4, CD8 (vpg34 and vpg9) (Willett et al., 1991), a murine monoclonal antibody against human IL-4 (1-41-1, Novartis, Vienna, Austria) (Roosje et al., 2002) and a polyclonal rabbit antibody against CD3 (AO452, DAKO, Dakopatts A/S, Glostrup, Denmark) (Day, 1998). Biotin-conjugated horse anti-mouse antibody (Vector, Burlingame, CA) or biotin-conjugated goat anti-rabbit antibody (Vector, Burlingame, CA) was used as a second step, followed by alkaline phosphate-conjugated streptavidin (Dakopatts A/S, Glostrup, Denmark). Staining was developed using naphthol AsBi phosphate (N-2250, Sigma, St. Louis, MO) as substrate and New Fuchsin as a chromogen, resulting in pink-red staining. Control staining was performed with an isotype-matched antibody (Becton Dickinson, San José, CA) and resulted in no staining. Sections were lightly counterstained with hematoxylin.

Positive staining cells in the superficial dermis were counted in four adjacent high power fields ($400\times$) and expressed as cells/ mm^2 . Cells in hair follicle walls and deep dermis were not included.

2.6. Statistical analysis

ANOVA for repeated measurements was used to compare the number of stained cells at different time points for both AD cats and control cats.

The Student's *t*-distribution test was used for comparison of pooled data of the three time points. A one-sample *t*-test was used to compare the pooled data of each marker staining for the group of AD cats with the control animals. A *P*-value of less than 0.05 was considered significant.

3. Results

3.1. Prick test

In the prick test, there was a concordance with the intradermal test results in three out of six tests for *D. farinae*, four out of six for *D. pteronyssinus*, three out of six for *T. putrescentiae*, and four out of six for the grass pollen mixture (Table 1). Despite the negative intradermal test in one AD cat, a reaction to grass pollen mixture was present in the prick test. This was considered relevant as clinical signs of AD were seasonal in this cat. The prick test and the intradermal test were negative in all control animals.

3.2. Atopy patch test

Three out of six AD cats had erythematous reactions with minimal induration to the applied allergens. Margins of these reactions were ill-defined and vesiculation or crusting was not observed. These were considered to be positive reactions. None of the control cats ($n = 10$) had positive reactions and no reactions were seen on the negative control site in the AD cats except for one reaction that was related to a clipper burn. Macroscopically, positive reactions appeared predominantly after 48 h. However, in one cat, positive reactions were already observed after 24 h (Table 2). Reactions occurred mainly with allergen concentrations of 100 000 and 25 000 NU/ml.

Table 1

Results of the intradermal test (idt) and prick test (pt) in cats with atopic dermatitis ($n = 6$)

	Allergen							
	Dfar		Dpt		Tyro		GP	
	idt	pt	idt	pt	idt	pt	idt	pt
Cat no. 1	+	–	+	+	–	–	–	–
Cat no. 2	–	+	–	+	+	+	–	+
Cat no. 3	–	–	–	–	–	–	–	+
Cat no. 4	+	+	+	+	–	+	–	–
Cat no. 5	+	–	–	–	+	–	–	–
Cat no. 6	+	+	+	–	+	+	–	–

Only allergens used in both tests are depicted (*Dermatophagoides pteronyssinus* (Dpt), *D. farinae* (Dfar), *Tyrophagus putrescentiae* (Tyro) and grass pollen mixture (GP)). (–), negative test result; and (+), positive test result.

Table 2

Results of the APT in AD cats with one or more positive reaction to aeroallergens

	Time after APT															
	24 h								48 h							
	Dpt		Tyro		Dfar		Gp		Dpt		Tyro		Dfar		GP	
	25	100	25	100	25	100	25	100	25	100	25	100	25	100	25	100
Cat no. 1	–	–	nd	nd	–	–	nd	nd	+	+	nd	nd	+	+	nd	nd
Cat no. 2	nd	nd	–	–	–	–	nd	nd	nd	nd	+	+	+	+	nd	nd
Cat no. 3 ^a	nd	nd	nd	nd	–	+	–	+	nd	nd	nd	nd	+	+	+	+

Cat no. 1 was tested with *Dermatophagoides pteronyssinus* (Dpt) and *D. farinae* (Dfar), cat no. 2 was tested with *Tyrophagus putrescentiae* (Tyro) and *D. farinae*, and cat no. 3 with *D. farinae* and grass pollen mixture (GP). nd, not done; (+), macroscopically positive; and (–), macroscopically negative. Values 25 and 100 are allergen concentrations (in $\times 10^3$ NU/ml).

^a Also had a positive APT reaction to a concentration of 5000 NU/ml of grass pollen mixture at 48 h.

One of these three AD cats reacted to the grass pollen mixture in a dilution of 5000 NU/ml at 48 h.

3.3. Immunohistochemistry

The results obtained with the immunohistochemistry staining are shown in Table 3 for the healthy control animals and Table 4 for the AD cats. Neither in the AD cats nor in the healthy control cats was there a significant difference in the negative control sites (i.e. NC_{10 h}, NC_{24 h}, NC_{48 h}) ($P > 0.05$) (ANOVA for repeated measurements).

There was also no significant difference in time (10, 24, and 48 h) in the number of stained cells at the allergen site corrected for the negative control value at that time point (e.g. Dp_{10 h}–NC_{10 h}), neither in the

group of the AD cats nor in the group of control animals (n.s.). For that reason, we pooled the results of the three time points, for each marker and allergen and for each cat. We then determined, for each marker and allergen, in the group of normal cats the mean and standard deviations and calculated (using Student's *t*-distribution) the 95% upper limit values in the group of normal cats. Values in animals exceeding these 95% upper limits were considered to be abnormal.

We found that one AD cat (no. 1) had a significant higher number of stained cells for IL-4, CD4, CD3, MHC class II, and CD1a at 24 h compared to the control animals (Table 3; one-sample *t*-test; values exceeding the 95% upper limit). This AD cat had macroscopically positive APT reactions for *D. farinae* and *D. pteronyssinus*. At 48 h APT this cat still

Table 3

Pooled number of cells/mm² per allergen (100 000 NU/ml) of five healthy control cats tested with Dpt and Dfar (APT-1) or Tyro and GP (APT-2) at 10, 24, and 48 h each corrected for the corresponding negative control values

	APT-1				APT-2				DC-1	DC-2
	Dfar		Dpt		Tyro		GP		Mean	Mean
	Mean	95%	Mean	95%	Mean	95%	Mean	95%		
IL-4	0.1	2	0.5	3	−0.2	0.5	−0.2	0.5	2	4
CD4	−1	8	1	11	−1	11	1	14	5	5
CD3	5	18	5	19	4	33	−1	21	11	13
CD8	4	21	2	17	0	6	0	5	7	25
MHC II	14	59	10	42	0	20	0	31	34	37
CD1a	−2	10	−1	10	−2	6	1	11	8	9

The first numbers in each column depict the mean values. In the second column, 95% upper limit values are depicted. DC-values are the means of the diluent control sites of the two groups of control cats (DC-1 (APT-1) and DC-2 (APT-2)), respectively.

Table 4

Number of positive-staining cells corrected for the negative control values after 24 h (first number per column) and 48 h patch testing with allergen concentrations of 100 000 NU/ml

Allergen	Patient	IL-4		CD4		CD3		CD8		MHC II		CD1a	
		24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Dfar	1 ^a	8	18	21	12	27	44	0	–8	59	46	60	9
Dfar	2 ^a	11	–1	3	16	12	59	0	–1	36	44	1	28
Dfar	3 ^a	1	3	6	–4	0	–11	2	–1	–14	91	5	8
Dfar	4	0	0	–12	–5	25	0	–1	0	7	–4	–10	–12
Dfar	5	6	1	6	–5	11	4	0	–1	3	49	–10	24
Dfar	6	5	9	2	1	–16	–2	2	–5	6	34	2	6
Dpt	1 ^a	10	8	17	7	39	32	0	–5	69	47	60	–12
Dpt	4	0	0	–7	4	23	15	–1	0	–19	21	–2	16
Tyro	2 ^a	1	–6	–1	22	5	32	0	–1	0	29	–2	13
Tyro	5	5	4	9	19	14	25	4	2	31	99	–3	56
Tyro	6	2	14	0	12	–7	9	3	0	–10	25	9	4
GP	3 ^a	2	1	7	2	8	–3	0	–2	44	59	12	–5

Dermatophagoides pteronyssinus (Dpt), *D. farinae* (Dfar), *Tyrophagus putrescentiae* (Tyro) or grass pollen mixture (GP). Significant values are written in italics.

^a Cats that had positive APT reactions.

showed a significant increase for these markers except for CD1a and MHC class II for the Dfar-APT. For the 48 h Dpt-APT a significantly increased number of cells staining positive for IL-4, CD3, and MHC class II were found. This cat also had positive APT reactions with Dpt at 25 000 NU/ml and Dfar at 25 000 NU/ml, both at 48 h APT. The cell numbers in the Dfar reaction were significantly increased for all markers except CD8 when compared to the control cats.

Because only AD cat no. 1 had significantly increased cell numbers with the allergen concentration of 25 000 NU/ml as well, cell numbers of positive APT reactions with the lower concentration are not shown in a table. At 24 h, Tyro-APT cells staining positive for IL-4 (cat no. 2) and IL-4 and MHC class II (cat no. 5) were significantly increased. At 48 h, Tyro-APT, AD cats no. 2 and 5 had significantly increased numbers of IL-4⁺ cells (only cat no. 5), CD4, MHC class II, and CD1a. In 24-h Tyro-APT skin of AD cat no. 6, significantly increased numbers of IL-4⁺ and CD1a⁺ cells were counted. At 48 h, the numbers of IL-4⁺, CD4⁺ and MHC class II⁺ cells were significantly increased. Skin of GP-APT AD cat no. 3 contained significantly more cells staining positive for IL-4, MHC class II and CD1a at 24 and 48 h (only IL-4 and MHC class II). In none of the APT reactions in the AD cats, we found a significant increase in the number of CD8⁺ cells.

4. Discussion

This report is the first to describe atopy patch testing in cats as an approach to study spontaneous AD in cats. In humans, the APT has been documented to be a very useful test in mimicking the natural disease process (Langeveld-Wildschut et al., 1996; Thepen et al., 1996). The reported number of positive reactions induced with this test in humans varies greatly (15–100%) and seems influenced by the method used (i.e. allergen concentration, tape stripping, allergens dissolved in liquid or petrolatum, and reading time) (Langeveld-Wildschut et al., 1995).

Tape stripping was used to remove superficial layers of the stratum corneum, thereby increasing the chance of allergen penetration, which may also happen spontaneously when cats lick themselves as part of their natural grooming behaviour or more likely when the licking has increased due to pruritus. Clipping and additional chemical epilation was necessary to ensure adhesion of the tape with Finn chambers.

We observed macroscopically positive APT reactions in three AD cats and in none of the healthy control animals. In addition, we did not find positive reactions towards the negative controls (diluent solution) in the AD cats. Hence, we conclude that the APT in cats can elicit positive reactions macroscopically. A

dilution of 100 000 NU/ml elicited more macroscopically positive reactions than lower allergen concentrations. In addition, the cellular infiltrate in the positive APT reactions evoked by low allergen concentrations was less prominent. We therefore conclude that a minimum concentration of 100 000 NU/ml is preferable. We did not observe, however, a consistent relationship between macroscopically positive reactions and cell numbers at the patch site. Like in cats of the present study, increased T cell numbers, however, have been described in macroscopically negative patch test sites in humans as well (Buckley et al., 1996).

An important point is the question whether positive reactions are evoked by a response to the allergen or by an irritant reaction. It has been reported in humans that purified house dust mite major allergens, Der p1 and Der p2, not only can be allergenic but also can have an additional proteolytic irritant effect, and induce irritant reactions in the APT (Deleuran et al., 1998). In addition, non-lesional skin in humans with AD (Uehara and Miyauchi, 1984) and cats with AD (Roosje et al., 1998, 2002) is histologically abnormal, rendering it more susceptible to irritant reactions.

The fact, however, that only few of the AD cats and not all of them reacted to the allergen application without reactions at the negative control site speaks in favour of a specific reaction. In addition, all of our healthy controls did not respond to the allergens which favours an allergic response.

We cannot exclude that the allergen solutions prepared from whole mite cultures may have contained such substances that could induce irritant reactions, although we have not found any documentation on irritant properties of *D. pteronyssinus*- or *D. farinae*-related enzymes in cat skin. Recently, it was reported that 68% of sera of AD cats containing antigen-specific IgE against *D. farinae* reacted with a *D. farinae* chitinase (McCall and Birmingham, 2000). We cannot exclude that this chitinase has a proteolytic irritant effect as well, causing a positive APT reaction. The positive APT reaction towards *D. farinae* in AD cat no. 3 that had only a positive prick test reaction towards grass pollen could have been caused by this proteolytic irritant effect. However, the significant increase in IL-4⁺ T cells in the APT in all AD cats except cat no. 4 is in support of a true allergic reaction. This is supported by the fact that in humans with AD it

has been reported that in irritant patch test reactions there is no increase of IL-4 mRNA (Grewe et al., 1995). Moreover, sensitivity to irritants is equal in AD patients having a positive or a negative APT response towards house dust mite (Langeveld-Wildschut et al., 2000).

The cellular infiltrate in the positive APT reactions was very similar to that in lesional skin of AD cats, i.e. an increase in IL-4⁺, CD4⁺, CD3⁺, MHC class II⁺, and CD1a⁺ cells (Roosje et al., 2002, 1998, 1997).

In contrast to the situation for the other markers, in none of the AD cats the number of CD8⁺ cells was significantly increased. The resulting increased CD4/CD8 cell ratio mimics the natural disease in cats and in humans (Markey et al., 1990; Roosje et al., 1998; Zachary et al., 1985). In the APT reaction in humans, a rapid increase in T cell numbers is seen until 48 h, reaching a maximum for the CD8⁺ cells at 24 h APT and for the CD4⁺ cells at 48 h APT. However, we did not observe a consistent increase in T cells comparing results at 24 h with those at 48 h.

In mice, percutaneous sensitisation with allergens through barrier-disrupted skin elicits a Th2-dominant cytokine response (Kondo et al., 1998). As there was no antibody available that is reactive with feline IFN- γ , we could not differentiate between a Th1, Th2 or Th0 response. Nevertheless, the finding of increased numbers of IL-4⁺ cells may indicate a Th2/Th0 response.

In humans, an increased number of CD1a⁺ cells, T cells and macrophages has been reported to occur in the dermis of positive and negative patch tests reactions (Sterry et al., 1991). Similar to these findings, we found significantly increased numbers of CD1a⁺ cells and MHC class II⁺ cells in both macroscopically positive APT sites and in negative APT reactions. We have no explanation for the high concentrations of MHC class II⁺ cells and the CD1a⁺ cells at the negative control sites of the control cats, although theoretically an occlusion artefact or a response to depilation may be responsible.

Summarising, we conclude that we were able to induce positive APT reactions in AD cats with a cellular infiltrate similar to the cellular infiltrate in lesional skin. Consequently, the APT is an appropriate tool to study the regulation of the inflammatory response in cats.

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