

Patch testing of experimentally sensitized beagle dogs: development of a model for skin lesions of atopic dermatitis

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Abstract

In humans with atopic dermatitis (AD), the epicutaneous application of allergens (atopy patch tests or APT) to which the patients are sensitized often results in the development of inflammation resembling that of spontaneous skin lesions. Dogs are affected with a natural homologue of human AD, but information on the induction of positive patch testing reactions is limited. The objectives of this pilot study were to determine the nature and cellular dynamics of inflammation occurring after APT in dogs hypersensitive to house dust mite and flea allergens. Laboratory Beagles were sensitized experimentally to *Dermatophagoides farinae* house dust mites (two dogs), *Ctenocephalides felis* flea saliva (one dog) or both (two dogs). Two other dogs served as nonsensitized controls. Both allergens and saline were applied epicutaneously. Macroscopic evaluations and skin biopsies were performed at 4, 24, 48 and 96 h after starting allergenic challenge. Biopsies were evaluated histologically and immunohistochemically with a panel of monoclonal antibodies specific for canine leucocyte antigens. Positive macroscopic reactions consisted of erythema, oedema and induration, and they occurred between 24 and 96 h after allergen application. Macroscopic and microscopic APT reactions developed only whenever serum IgE was present against tested allergens. Microscopically, positive APT was associated with epidermal hyperplasia, Langerhans' cell hyperplasia, and eosi-

nophil and lymphocyte epidermotropism. Dermal inflammation was mixed and arranged in a superficial perivascular to interstitial pattern. Numerous IgE⁺-CD1⁺ dendritic cells and gamma-delta T-lymphocytes were observed. Macroscopically and microscopically, APT reactions in these experimentally sensitized animals resembled those seen in lesional biopsy specimens of dogs and humans with spontaneous AD. Therefore, APT in hypersensitive dogs provides a relevant experimental model to investigate the pathogenesis and treatment of both canine and human AD skin lesions.

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Introduction

In dogs, atopic dermatitis (AD) is a common affliction that has been redefined recently as a 'genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features that is associated most commonly with IgE antibodies to environmental allergens'.¹ Canine and human AD exhibit a remarkable similarity at clinical, immunopathological and therapeutic levels.^{2,3} In both species, genetic factors underlie the disease, allergen-specific IgE antibodies are detectable in the majority of patients and skin lesions appear to proceed from IgE-dependent immediate and late-phase reactions.³ Primary lesions of canine and human AD include erythematous macules, patches and papules, and these evolve into secondary excoriated, lichenified and scaly lesions because of intractable scratching, chewing and rubbing presumably caused by underlying pruritus. In adult patients of both species, the distribution of lesions appears to be similar with the involvement of face, hands and feet (or paws), flexural aspects of extremities and/or folded areas subjected to friction.^{3,4} Drugs with good evidence of efficacy for reducing signs of canine and human AD include topical or oral glucocorticoids and the calcineurin inhibitors tacrolimus or cyclosporin.^{5,6}

In recent decades, studies on the mechanisms of development of AD skin lesions have relied on the comparison of many parameters (e.g. cell surface molecules, cytokines, chemokines, etc.) in samples of lesional or non-lesional skin collected from affected patients. Unfortunately, such approaches are inherently limited by the caveat that, even though lesions may be categorized as 'acute' or 'chronic', there is an uncertainty regarding the specific 'immunological age' of the lesion sampled. Indeed, the latest contact with offending allergens could have occurred minutes, hours or days before that particular

site was biopsied. The unknown age of the lesion could have a noticeable impact on the interpretation of the immunological phenomena recorded, as, for example, the cytokine repertoire secreted in the skin of human AD is known to vary with the duration of lesions.⁷ In contrast, deliberate experimental sensitization and challenge in a laboratory setting allow the lesion age, plus many other variables, to be predetermined. The spectrum of allergens to which the animal is exposed, differences in potentially exacerbating factors such as infections, parasite infestations and diet can all be controlled in the laboratory.

To alleviate the concern of uncertain lesion age, there is a need for experimental models that could reproduce the immunological sequence of AD skin lesions. Several approaches have been pursued. For example, intradermal injections of relevant allergens or polyclonal anti-IgE antibodies into normal or atopic dog skin reproduce immediate and late-phase reactions that mimic dermal but not epidermal microscopic lesions of canine AD.^{8,9} In humans with AD, the epicutaneous application of allergens to which the patient is hypersensitive ('atopy patch test', APT) reproduces most, if not all, immunological changes seen in natural lesions.¹⁰ Either of these methods is also valuable to test the efficacy of anti-allergic drugs for the prevention or treatment of established allergen-induced lesions. Prior to this study, there has been only one small article describing the performance of APT in normal and atopic dogs, and results were inconsistent from dog to dog.¹¹

The objective of this paper is to report the induction of hypersensitivity and development of positive APT in beagles. The remarkable similarity between APT and natural canine and human AD skin lesions makes this experimental model very useful for studying the immunopathogenesis of AD skin lesions or for screening drugs for prevention or treatment of this disease in either species.

Materials and methods

Canine subjects

Two IgE-hyper-responsive beagles (dogs 1 and 2) were obtained from Lovelace Research Institute (Albuquerque, New Mexico), and five beagles with no documented genetic predisposition to IgE hyper-responsiveness were purchased from Marshall Farms (dogs 3–7). There were four intact males and three females, one of which was spayed. At the time of patch testing, the age of the dogs ranged from 2.2 to 5.3 years (median: 5.2 years).

Sensitization

In this study, a successful sensitization was defined as the induction of elevated serum levels of allergen-specific IgE. Dogs 1 and 2 were sensitized to *Dermaphagoides farinae* house dust mite (Df-HDM) by repeated subcutaneous injections of 10 µg of Df-HDM (Greer Laboratories, Lenoir, NC) in 1 mg alum, every 3 weeks from 10 days to 6 months of age. Dogs 3–5 were sensitized to *Ctenocephalides felis* flea saliva (Cf-FS) by the application of flea-containing chambers on their thorax for 20 min once weekly for 40 weeks. One year before this study, dogs 3 and 4 became sensitized to Df-HDM after intradermal injections and epicutaneous application (patch testing) as part of an experiment the previous year.¹² Finally, dogs 6 and 7 were not sensitized to either Df-HDM or Cf-FS, and they therefore served as negative controls.

Allergen-specific IgE serology

In each of the seven dogs, serum was collected for determination of allergen-specific IgE using an enzyme-linked immunosorbent assay that employs, for specific IgE detection, biotinylated recombinant

extracellular alpha chain of human high-affinity IgE receptors (FcεRIα). This test has been validated for canine use, and is available commercially (ALLERCEPT® Definitive Allergen Panels, Heska Corporation, Ft Collins, Colorado).¹³ Serum for allergen-specific IgE serology testing was obtained before patch testing and 10 days afterwards. All sera were tested at 1 : 10 and serial threefold dilutions to 1 : 2430. Results were expressed as optical density (OD) × 1000. At the 1 : 10 dilution, values of 100 or greater were considered positive.

Patch testing and skin biopsy collection

For patch testing, the Df-HDM allergens were applied as approximately 100 µL of a paste of whole *D. farinae* mites (Heska Corp, Ft Collins, CO) made from 1 g of mites sonicated in 1.5 mL of phosphate-buffered saline. The Cf-FS allergens consisted of 50 µL of flea saliva (953 µg mL⁻¹) collected as described previously.¹⁴ Fifty microlitres of saline served as an inert control.

One day before testing, a wide patch of skin was clipped on the lateral thorax of each dog. Patch test areas were marked with an indelible felt pen. Four sets of patches containing Df-HDM, Cf-FS allergens and saline were deposited onto 1-cm-wide square adhesive bandages (Band-Aid® Clear Spot, Johnson & Johnson Consumer Products Company, Skillman, New Jersey) that were stuck firmly to the skin, covered in gauze and held in place with surgical tape and wrapping bandage (Vetrap, 3M, St Paul, Minnesota). Each dog wore a mesh body suit (Alice King Chatham Medical Arts, Hawthorne, California) to prevent removal of the patch and further self-trauma.

Four, 24, 48 and 96 h after applying the allergens onto the dogs' skin, punch biopsies of one set of each patch test series were obtained after appropriate local anaesthesia with lidocaine. Biopsies were bisected immediately. One-half of each specimen was placed in neutral buffered formalin for routine embedding in paraffin. The other half was deposited in Optimal Cutting Temperature medium (OCT Tissue Tek, Baxter Diagnostics Inc., McGaw Park, Illinois), immersed in isopentane cooled to its freezing point in liquid nitrogen, and then stored at -70 °C until processed for immunohistochemical staining.

For brevity and clarity, the 'relevant allergen' designation will be used to describe an allergen to which the dog was sensitized (e.g. Df-HDM in dogs 1–4; Cf-FS in dogs 3–5), and 'irrelevant allergen' will be employed for reactions and samples collected at sites challenged with allergens to which the dogs was not sensitized (e.g. Cf-FS in dogs 1, 2, 6 and 7, Df-HDM in dog 5–7).

Macroscopic lesional scores

Scoring of macroscopic skin lesions was performed 4, 24, 48 and 96 h after beginning the application of allergens onto the dogs' skin. A four-point scale was used as follows: -, no visible reaction; +, erythema; ++, erythema and induration or oedema (papules); +++, erythema with vesiculation or more severe reactions.

Histopathology

Five-micrometre sections were cut from paraffin blocks and stained with routine haematoxylin-eosin for visualization of the inflammatory reaction pattern. Additional paraffin sections were coloured with Luna's stain for eosinophils.¹⁵

In haematoxylin-eosin-stained skin sections, images of the entire dermal area were acquired with a video camera. Using a morphometric software (Image Pro Plus, MediaCybernetics, San Diego, California), the dermal area in each image was determined after exclusion of sites of vasculature and adnexae. Cell numbers were tallied after selection of each individual dermal cell. Dermal counts were reported as number of cells per mm² of dermal sectional area. After examination of Luna-stained paraffin sections, eosinophils were recorded as being absent or present (scattered or clustered) in the epidermis. In the dermis, the percentage of eosinophils was graded as follows: 0, none; 1, < 5% of dermal cells; 2, 5–33% of dermal cells; 3, 34–66% of dermal cells; and 4, > 67% of dermal cells.

Immunohistochemistry

To determine the phenotype of dermal and epidermotropic mononuclear cells, a three-step labelled streptavidin method was

used.¹⁶ Primary monoclonal antibodies specific for the canine leucocyte antigens CD1c, CD3,¹⁷ CD4, CD8alpha and the alpha-beta¹⁸ and gamma-delta T-cell receptors¹⁹ were obtained from Dr P.F. Moore (University of California Davis). All were used at 1 : 10 dilution. The anticanine IgE monoclonal antibody 5.91 was a gift by Dr B. Hammerberg (NC State University, Raleigh, North Carolina).²⁰ Double immunostaining with antibodies directed against canine CD1c and IgE was performed using rabbit anticanine IgE polyclonal antiserum (B. Hammerberg, NC State University), visualized using a two-step immunoperoxidase method and a red chromogen, as well as monoclonal anticanine CD1c stained with a two-step alkaline phosphatase-based technique and a blue chromogen.

The nature and phenotype of epidermal and dermal mononuclear cells (dendritic antigen-presenting cells, T lymphocytes) were assessed qualitatively, and the percentage of dermal cells was rated as for dermal eosinophils as described previously.

After preliminary studies established that the epicutaneous application of saline did not lead to the dermal or epidermal emigration of granulocytes or mononuclear cells (dermal scores < 1 for all antibodies), the percentage of dermal cells immunostained for each marker was not graded in control patch tests.

Statistics

For all statistical analyses, two-tailed *P* values were calculated, parametric tests were used after verification of normality of data sets, and the level of significance was set to 5%.

Dermal cell counts at the various time points were compared within groups (relevant allergens, irrelevant allergens, saline) by means of repeated-measures ANOVA followed by Tukey's multiple comparison post-tests. For comparison between groups at each time point, unpaired *t* tests with Welch's correction were used. Correlations between dermal cell counts and serum IgE levels were assessed by determination of Spearman's correlation coefficient. The statistical software used was Prism 4.0 (GraphPad Software, San Diego, California).

Results

Allergen-specific IgE serology

In half of mite-sensitized and all flea-sensitized dogs, IgE antibody levels were present against relevant allergens before patch testing (Table 1). Dogs 3 and 4 had high levels of IgE against Df-HDM the previous year, but lack of challenge with these allergens for a year led to a decrease in HDM-specific IgE titres until this study. Indeed, repeated evaluation 10 days after APT revealed high serum IgE against relevant allergens in all sensitized dogs (Table 1). The two control beagles were negative for Df-HDM or Cf-FS-specific serum IgE when 100 was considered the cut-off value.

Table 1. Allergen-specific IgE serology before and after patch testing

Dogs	Exposure	Df-HDM		Cf-FS	
		Before APT	After APT	Before APT	After APT
1	Df-HDM	2064	2850	70	74
2	Df-HDM	2435	4061	0	0
3	Cf-FS + Df-HDM	66	3179	1698	2173
4	Cf-FS + Df-HDM	7	553	460	427
5	Cf-FS	0	0	102	373
6	none	24	22	0	36
7	none	16	0	36	0

Abbreviations: Cf-FS: *Ctenocephalides felis* flea saliva, Df-HDM: *Dermatophagoides farinae* house dust mites.

Note: values in bold are considered positive (> 100 EA units).

Macroscopic lesional scores

Results of lesional scores are available in Table 2. Four hours after the application of mite or flea allergens, skin lesions were not visible. Twenty-four hours after the onset of the test, erythema, with or without induration or oedema (papules), developed at the site of relevant allergen application in 3/4 mite- and 2/3 flea-sensitized dogs. Forty-eight hours after Df-HDM and Cf-FS allergen application, similar reactions were observed in 3/4 and 3/3 dogs, respectively. After 96 h, positive reactions to these allergens were discovered in 2/4 and 2/3 dogs, respectively. In contrast, macroscopic reactions did not develop at the site of saline and irrelevant allergen application, nor was any reaction visible in the patch tests performed on nonsensitized dogs 6 and 7.

Histopathology

At sites of challenge with relevant allergens, dermal cellularity increased significantly during the course of the study (repeated measures ANOVA; *P* = 0.0024) (Table 3). Counts at 96 h were significantly higher than those at 4, 24 and 48 h (Tukey's tests; *P* < 0.05). The main inflammatory pattern consisted of varying degrees of superficial perivascular to interstitial mononuclear dermatitis with eosinophils. Epidermal lesions included irregular hyperplasia and multifocal lymphocyte and eosinophil exocytosis (Fig. 1). Occasionally, eosinophils clustered to form microabscesses. In contrast, patch tests with irrelevant allergens or saline did not lead to significant elevations in dermal cell counts (Table 3).

Table 2. Lesional scores during patch testing

Dogs	Exposure	4 h			24 h			48 h			96 h		
		Df-HDM	Cf-FS	Saline	Df-HDM	Cf-FS	Saline	Df-HDM	Cf-FS	Saline	Df-HDM	Cf-FS	Saline
1	Df-HDM	–	–	–	++	–	–	+	–	–	++	–	–
2	Df-HDM	–	–	–	+	–	–	+	–	–	–	–	–
3	Cf-FS + Df-HDM	–	–	–	++	–	–	++	+	–	–	–	–
4	Cf-FS + Df-HDM	–	–	–	–	+	–	–	++	–	+	++	–
5	Cf-FS	–	–	–	–	+	–	–	+	–	–	+	–
6	None	–	–	–	–	–	–	–	–	–	–	–	–
7	None	–	–	–	–	–	–	–	–	–	–	–	–

Abbreviations: Cf-FS: *Ctenocephalides felis* flea saliva; Df-HDM: *Dermatophagoides farinae* house dust mites.

Grading: (+) erythema; (++) erythema and induration or edema (papules); (+++) erythema with vesiculation or more severe reactions.

Table 3. Dermal cell counts during patch testing

Allergens	4 h	24 h	48 h	96 h
Relevant	359 (270–449)*	492 (259–726)†	569 (366–773) ‡§¶	1370 (577–2164)*†‡§¶**††
Saline	300 (235–365)	291 (230–353)	351 (264–438)¶	343 (245–441)††
Irrelevant	337 (246–427)	363 (231–496)	343 (268–418)§	396 (343–450)**

Data presented are means (95% confidence intervals).

Values sharing the same symbol are significantly different as follows: *†: $P < 0.01$; ‡§¶**††: $P < 0.05$.

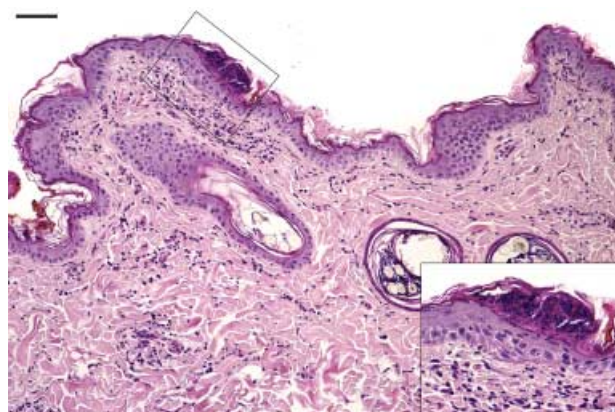


Figure 1. Histological staining of patch tests with relevant allergens. Dog 1: 24 h after relevant allergen challenge (Df-HDM), there is epidermal hyperplasia, superficial dermatitis and crusting arising from a drying subcorneal pustule (inset). H&E; bar = 75 µm.

At 48 and 96 h, cell numbers at sites challenged with relevant allergens were significantly higher than those where irrelevant allergens or saline were applied (unpaired t tests; $P < 0.05$). There were no significant differences, at any time point, between cell counts of samples collected at irrelevant allergen or saline-challenged sites.

All skin sections were stained with Luna's stain to assess eosinophil infiltration. In skin biopsies collected 4 h after the onset of allergen challenge, only very rare eosinophils were detected. From 24 to 96 h, epidermal eosinophils were detected in 3/5 and 0/7 dogs challenged with relevant and irrelevant allergens, respectively. In the three dogs where eosinophils were observed, cells were clustered in microabscesses. During the same time period, dermal eosinophils were observed in all dogs at sites challenged with relevant allergens. The maximal grade for eosinophil dermal infiltration was 2 (5–33% of dermal cells).

Immunohistochemistry

Tissue infiltration with epidermal and dermal dendritic cells was assessed by immunohistochemical staining using monoclonal antibodies specific for canine CD1c. Epidermal Langerhans' cells (LC) expressing CD1c were observed in all skin samples collected from normal and hypersensitive dogs, whether they were challenged with relevant or irrelevant allergens or saline. After 24 h following relevant allergen challenge, LC tended to become highly dendritic and clustered (Figs 2 and 3a), in contrast to being scattered and mildly dendritic at sites challenged with irrelevant allergens (Fig. 3a). Infiltration of CD1c⁺ dendritic cells increased progressively during the study period at sites challenged with relevant allergens, and

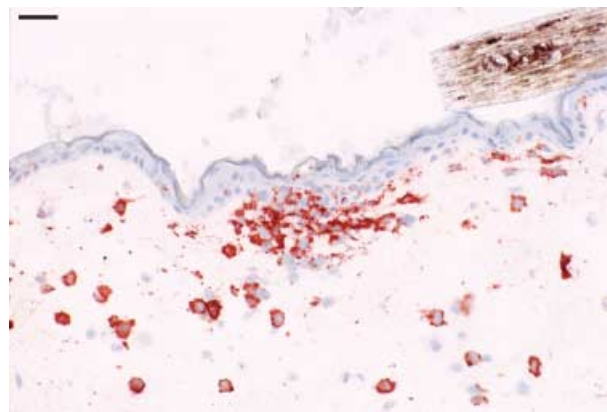


Figure 2. Immunostaining for CD1c of patch tests with relevant allergens. Dog 1: 48 h after relevant allergen challenge (Df-HDM), there is hyperplasia of dermal dendritic cells, which cluster in the superficial dermis. Immunohistochemistry; CD1c monoclonal antibody 9H11; AEC chromogen with haematoxylin counterstain; bar = 35 µm.

cells often became larger and formed large clusters or diffuse spreads. Immunostaining for IgE revealed positive cells in both epidermis and dermis (Fig. 3b). Double immunostaining for CD1c and IgE revealed IgE⁺ dendritic cells, often in high numbers, in the dermis of all dogs hypersensitive to Df-HDM at sites challenged with these allergens (Fig. 4). In normal dogs and in those hypersensitive to Cf-FS, such CD1c-IgE double-positive dermal cells, were not detected at sites challenged with this allergen (data not shown).

During the study period, T lymphocytes expressing CD3 were detected in the epidermis of all dogs at sites challenged with relevant allergens. Focal epidermal clusters were observed. In the dermis, lymphocytosis was highest after 96 h, and it was strongest at sites challenged with relevant allergens (Fig. 5). Most lymphocytes stained positively for the alpha-beta T-cell receptor. Remarkably, T lymphocytes expressing the gamma-delta T-cell receptor were detected in the epidermis of skin biopsies of 4/5 dogs challenged with relevant allergens (Fig. 6). In contrast, such cells were not observed in the epidermis of samples collected at sites where irrelevant allergens were applied. Rare gamma-delta T-lymphocytes were found in the dermis of most skin biopsies. In the epidermis and dermis, T-lymphocytes expressed either CD4 or CD8, and for most biopsies, dermal infiltration scores were usually one grade higher for CD4 compared to CD8 cell counts.

Discussion

In this paper, we report the successful sensitization to *Dermatophagoides* and flea salivary antigens of IgE

Figure 3. Immunostaining for CD1c and IgE of patch tests with relevant allergens. Dog 1: 48 h after relevant allergen challenge (Df-HDM), Langerhans cells are hyperplastic and aggregate in the epidermis (a). Immunostaining of a matching section reveals that several cells in the same epidermal cluster express IgE (b). Immunohistochemistry; CD1c monoclonal antibody 9H11 (a); IgE monoclonal antibody 5.91 (b); AEC chromogen with haematoxylin counterstain; bar = 100 µm.

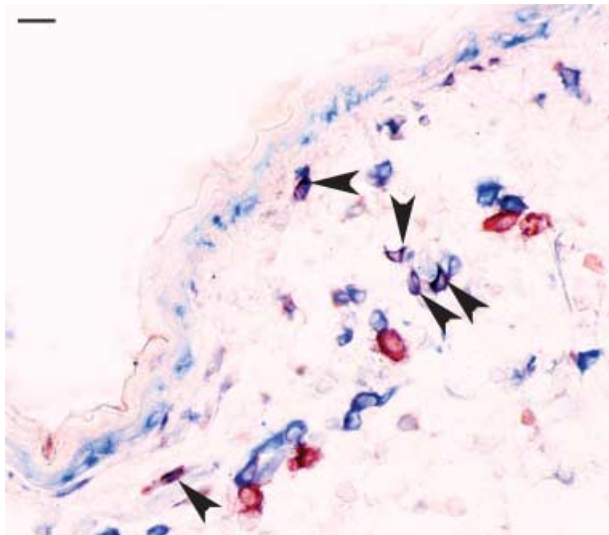
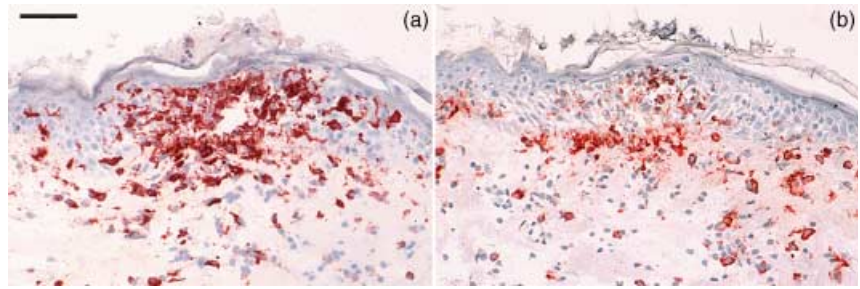


Figure 4. Double immunostaining for ige-positive dendritic cells. Dog 3: 48 h after relevant allergen challenge (Df-HDM), there are scattered dermal dendritic cells that express both IgE (red) and CD1c (blue) chromogens and appear black (arrowheads). Epidermal Langerhans cells and other dermal dendritic cells are blue (CD1c-positive) whereas dermal mast cells are red (IgE-positive). Immunohistochemistry; anti-CD1c monoclonal antibody 9H11 and anti-IgE polyclonal antiserum; no counterstain; bar = 20 µm.

hyperresponsive and normal laboratory Beagles. In these dogs, epicutaneous (patch test) application of allergens elicited macroscopic and microscopic reactions whenever the subject had elevated serum IgE against applied allergens. Gross and microscopic reactions mirrored those of skin lesions seen in dogs and humans with AD, suggesting that this model would be valuable to study disease pathogenesis or preclinical treatment modalities.

In this study, macroscopic reactions, which consisted of erythematous macules (grade: +) with or without papules (grade: ++), were observed from 24 to 96 h after epicutaneous challenge with relevant allergens. The highest lesional scores occurred at the 48 h time point. The intensity of macroscopic reactions after APT was delayed compared to a previous report of late-phase reactions (LPR) induced in the skin of allergic dogs after intradermal injections of Df-HDM.⁸ In intradermally induced LPR, highest clinical scores are present at 6 and 12 h evaluation end points.⁸ This time difference between the two challenge methods is likely caused by the delay needed for allergen capture, processing and induction of vasoactive and cellular responses after epicutaneous provocations compared to intradermal allergen provocations. Nevertheless,

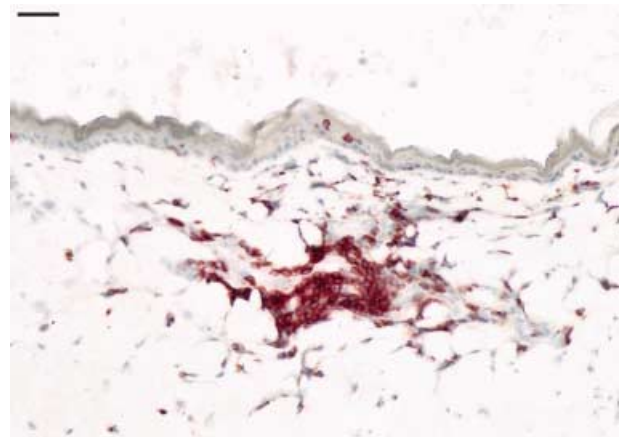


Figure 5. Immunostaining for CD3 of patch tests with relevant and irrelevant allergens. Dog 1: 48 h after relevant allergen challenge (Df-HDM), there is infiltration of T lymphocytes, which have aggregated in a subepidermal location. Scattered T lymphocytes are also found in the epidermis. Immunohistochemistry; CD3 monoclonal antibody 2A12; AEC chromogen with haematoxylin counterstain; bar = 50 µm.

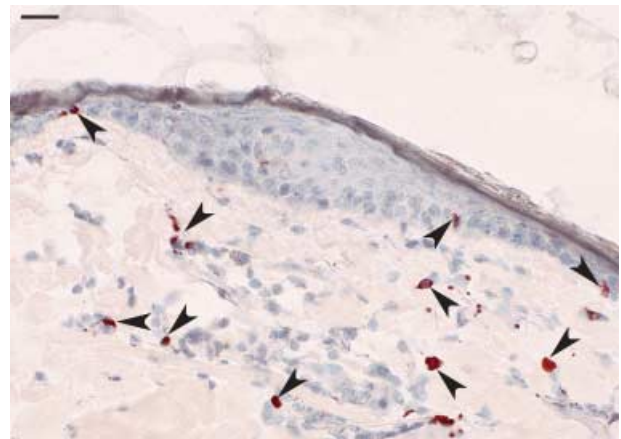


Figure 6. Immunostaining for gamma-delta T-cell receptor of patch tests with relevant allergens. Dog 2: 48 h after relevant allergen challenge (Df-HDM), there are scattered T lymphocytes expressing the gamma-delta T-cell receptor in the dermis and the epidermis (arrowheads). Immunohistochemistry; monoclonal antibody 8H1; AEC chromogen with haematoxylin counterstain; bar = 25 µm.

the high scores seen when reading APT in these dogs 48 h after allergen challenge are consistent with the latest recommendations of the European Task Force on Atopic Dermatitis for APT evaluation in humans with AD.²¹ Whether an additional reading of canine APT 72 h after challenge

would be beneficial compared to the 48-h time point is unknown.

In this study, positive macroscopic APT reactions developed only whenever dogs had serum IgE against allergens used for provocation (i.e. 'relevant allergens'). The application of allergens to which the dogs were not sensitized (i.e. 'irrelevant allergens') was not followed by visible inflammation. Similarly, results from a recent study confirmed that positive APT with HDM mixtures only developed in atopic dogs with positive immediate intradermal reactions against these allergens.²⁵ Altogether, these observations provide indirect evidence supporting the hypothesis that allergen-specific IgE antibodies could be involved in the generation of APT reactions in the dog.

In human patients with AD, however, APT reactions appear to develop both in the presence or in the absence of corresponding allergen-specific IgE. On one hand, results from a randomized multicentre study suggested that APT reactions were concordant with those of skin prick and RAST allergen-specific IgE serological tests.²² In contrast, positive APT reactions have been elicited on the skin of human patients with 'intrinsic' AD,^{23,24} a disease variant where elevated total and allergen-specific IgE are not found. In the latter subset of AD, the influx of inflammatory dendritic epidermal cells appears to be an early event that might be relevant to disease pathogenesis.²⁴ As in this canine study, cutaneous inflammation was seen only whenever serum IgE was elevated against applied allergens, APT in dogs may be more relevant for modelling 'extrinsic' rather than 'intrinsic' AD skin lesions of humans.

In these seven beagles, the epicutaneous application of saline or 'irrelevant' allergens did not lead to any noticeable rise in total dermal cell counts throughout the duration of this study. In contrast, the application of 'relevant' allergens—those against which the dogs had high serum IgE levels—led to a marked increase in dermal cell numbers. In this study, the 95% confidence interval of the dermal cellularity was nearly identical to the dermal cell counts of clinically normal (mean: 780 cells per mm²) and lesional canine AD skin (mean: 2661 cells per mm²).¹⁶ Overall, the pattern of inflammation after APT with 'relevant' allergens was similar to that seen in natural lesional AD skin in dogs.¹⁶ Indeed, in both positive APT and lesional canine AD skin, epidermal lesions consisted of irregular epidermal hyperplasia with lymphocyte and eosinophil exocytosis occasionally forming subcorneal microabscesses.¹⁶ Additionally, dermal lesions were arranged in a superficial perivascular to interstitial inflammatory pattern (e.g. scattered dermal inflammation).¹⁶ These observations are similar to those reported in the recently published study.²⁵ Of note is that the often chronic nature of canine AD skin lesions causes more pronounced epidermal hyperplasia than that seen in APT in this study, which lasted only 96 h.

Remarkably, the dermal inflammation seen after positive APT was reminiscent to that seen after intradermal LPR in dogs. However, there were epidermal changes after APT, whereas none were observed during intradermally induced LPR.⁸ Of the APT epidermal lesions that might be most relevant to the pathogenesis of AD, LC hyperplasia with cluster formation and eosinophil epidermotropism with subcorneal abscess formation were the most noticeable. Indeed, these lesions are among the most

characteristic microscopic changes seen in canine diseased AD skin,^{16,26} but these are notably absent from LPR induced in the dermis of atopic dogs.⁸

One of the most unique features of canine AD skin inflammation is the presence of T lymphocytes expressing the gamma-delta T-cell receptor.¹⁶ This observation has been used to support the hypothesis that some relevant allergen contact with immune cells occurs in the epidermis of dogs with AD.²⁷ This finding, which is rare among canine dermatoses,²⁸ is not observed in human AD skin lesions.²⁹ Such species difference could be the marker of a variation in cutaneous epithelial defence between humans and dogs.

Remarkably, gamma-delta receptor-expressing T lymphocytes were found in most biopsies from APT performed with 'relevant' allergens in this study, whereas none were noticed in skin biopsies performed after intradermal allergen challenges reported previously.⁸ It is logical to hypothesize that although epicutaneous allergen challenge might lead to the recruitment of gamma-delta T lymphocytes—cells involved in epithelial defence, intradermal challenge with similar allergens would not.

Finally, performance of APT with *Dermatophagoides* allergens in mite-hypersensitive beagles reproduced a key feature of atopic cutaneous inflammation: the presence of IgE⁺ dendritic cells. Such IgE-expressing dendritic cells are found in lesional skin biopsies of both dogs²⁶ and humans^{30,31} with AD, and it is thought that IgE at the surface of dendritic cells are involved in both allergen capture and focused presentation.^{32,33} Of interest is the lack of observation of IgE⁺ dendritic cells at sites challenged with flea saliva in flea-sensitized dogs. This difference between mite and flea allergen challenges might be caused by the chemical composition of the allergen(s) used or, possibly, to a variable immune response to allergens that are naturally found on the skin surface (mites) or injected directly into the dermis (flea saliva).

In conclusion, in spite of its limitation because of small number of tested subjects, this study of APT in experimentally sensitized beagle dogs provides preliminary evidence that positive reactions occur whenever IgE serum levels are high against challenged allergens. Moreover, positive APT reactions reproduce most, if not all, macroscopic and microscopic changes seen in acute skin lesions of canine and human AD. Therefore, this experimental model offers a unique opportunity to study the pathogenesis of this disease and to test new treatment modalities.

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Résumé Chez les patients humains souffrant de dermatite atopique (AD), l'application épicutanée d'allergènes (atopy patch tests, APT) auxquels le patient est sensibilisé provoque une inflammation ressemblant à celle observée dans les lésions spontanées. Les chiens sont affectés par une maladie qui ressemble à celle de l'homme, mais les données relatives aux tests épicutanés sont encore parcellaires dans l'espèce canine. Les buts de cette étude pilote étaient de déterminer la nature et la dynamique cellulaire de l'inflammation apparaissant après APT chez des chiens sensibilisés aux acariens des poussières et aux puces. Des Beagle de laboratoire ont été sensibilisés expérimentalement à *Dermatophagoides farinae* (deux chiens), la salive de *Ctenocephalides felis* (un chien) ou les deux (deux chiens). Deux autres chiens ont servi de contrôle. Les allergènes et du soluté salé ont été appliqués par voie épicutanée. Des évaluations macroscopiques et des biopsies cutanées ont été réalisées à 4, 24, 48 et 96 heures après le début de la stimulation allergénique. Les biopsies ont été étudiées sur le plan histologique et sur le plan immunohistochimique, avec un ensemble d'anticorps monoclonaux spécifiques des antigènes leucocytaires canins. Des réactions macroscopiques positives consistaient en un érythème, un œdème et une induration, et apparurent entre 24 et 96 heures après application des allergènes. Des réactions macroscopiques et microscopiques n'étaient observées

qu'en présence d'IgE sériques contre les allergènes testés. Sur le plan microscopique, des APT positives étaient caractérisées par une hyperplasie épidermique, des cellules de Langerhans, et un épidermotropisme des éosinophiles et des lymphocytes. L'inflammation dermique était mixte et s'organisait selon un patron périvasculaire ou interstitiel. De nombreuses cellules dendritiques IgE⁺-CD1⁺ et des T-lymphocytes gamma-delta étaient observés. Macroscopiquement et microscopiquement, les réactions APT observées chez ces chiens sensibilisés ressemblent à celles observées dans les lésions spontanées d'AD du chien ou de l'homme. C'est pourquoi les APT chez les chiens hypersensibilisés représentent un modèle expérimental possible pour étudier la pathogénie et le traitement des lésions d'AD chez le chien et l'homme.

Resumen En personas con dermatitis atópica (DA), la aplicación epicutánea de alérgenos (prueba del parche para atopía, PPA) para los cuales los pacientes están sensibilizados, a menudo resulta en un proceso inflamatorio similar a las lesiones espontáneas. Muchos perros son afectados por un proceso equivalente al humano, pero la información relativa a la inducción de reacciones positivas a la prueba del parche es limitada. Los objetivos de este estudio piloto fueron determinar la naturaleza y dinámica celular de la reacción inflamatoria a la PPA en perros sensibilizados a alérgenos del ácaro del polvo y de pulgas. Perros Beagle de laboratorio fueron sensibilizados experimentalmente al ácaro del polvo *Dermatophagoides farinae* (dos perros), y a saliva de pulgas (*Ctenocephalides felis*) (un perro) o a ambos (dos perros). Otros dos perros sirvieron como controles no sensibilizados. Ambos alérgenos y suero salino fueron aplicados por vía epicutánea. La evaluación macroscópica y de las biopsias fue realizada a las 4, 24, 48 y 96 horas tras la reexposición al alérgeno. Las biopsias se evaluaron mediante histología e inmunohistoquímica con un panel de anticuerpos monoclonales específicos para antígenos leukocitarios caninos. Las reacciones clínicas positivas incluyeron eritema, edema e induración y ocurrieron entre las 24 y 96 horas tras la aplicación del alérgeno. Reacciones a la PPA macroscópicas y microscópicas se observaron solo cuando hubo producción de IgE frente al alérgeno. En el examen microscópico, una reacción PPA positiva estuvo asociada con hiperplasia de la epidermis, hiperplasia de células de Langerhans, y epidermotropismo de los linfocitos y eosinófilos. La reacción en la dermis fue de tipo mixto, con un patrón perivascular e intersticial superficial. Se observaron numerosas células dendríticas IgE⁺-CD1⁺ y linfocitos T-γδ. Las reacciones macroscópicas y microscópicas en estos perros fueron similares a las observadas de forma espontánea en perros y humanos con dermatitis atópica. Por lo tanto, la PPA en perros sensibilizados representa un modelo experimental de relevancia para estudiar la patogénesis y el tratamiento de las dermatitis atópicas canina y humana.

Zusammenfassung Bei Menschen mit atopischer Dermatitis (AD) resultiert die epikutane Applikation von Allergenen (Atopie Patch Test, APT), auf welche die Patienten sensibilisiert sind, oft in der Entstehung einer Entzündung, die den spontanen Hautläsionen ähnelt. Hunde sind von einer der menschlichen AD naturgemäß entsprechenden Krankheit betroffen, aber die Information bzgl. der Induktion von positiven Patch Test Reaktionen ist limitiert. Die Ziele dieser Pilotstudie bestanden darin, die natürliche und zelluläre Dynamik der Entzündung zu bestimmen, die nach APT bei Hunden auftritt, die überempfindlich auf Hausstaubmilben- und Flohallergene sind. Labor-Beagles wurden experimentell auf *Dermatophagoides farinae* Hausstaubmilben (zwei Hunde), *Ctenocephalides felis* Flohspeichel (ein Hund) oder beides (zwei Hunde) sensibilisiert. Zwei weitere Hunde dienten als nicht-sensibilisierte Kontrolltiere. Beide, Allergene und Kochsalzlösung, wurden epikutan appliziert. Eine makroskopische Beurteilung, sowie die Entnahme von Hautbiopsien, wurde 4, 24, 48 und 96 Stunden nach Beginn der allergischen Provokation durchgeführt. Die Biopsien wurden histologisch sowie immunhistochemisch mittels einer Reihe von monoklonalen Antikörpern, die canines Leukozyten Antigen spezifisch binden, beurteilt. Positive makroskopische Reaktionen bestanden aus Erythem, Ödem und Induration, die zwischen 24 und 96 Stunden nach der Allergenapplikation auftraten. Makroskopische und mikroskopische APT Reaktionen entstanden nur, wenn jeweils auch Serum-IgE gegen die Testallergene vorhanden waren. Mikroskopisch wurden positive APT assoziiert mit epidermaler Hyperplasie, Langerhanszell-Hyperplasie, sowie Epidermotropismus von Eosinophilen und Lymphozyten. Die dermale Entzündung war gemischt und in einem oberflächlichen perivaskulären bis interstitiellen Muster angeordnet. Zahlreiche IgE-CD1⁺ dendritische Zellen und gamma/delta T-Lymphozyten wurden gefunden. Makroskopisch und mikroskopisch waren die APT Reaktionen bei diesen experimentell sensibilisierten Tieren ähnlich wie die der Biopsien von Hautveränderungen bei Hunden und Menschen mit spontaner AD. Daher stellt der APT bei überempfindlichen Hunden ein bedeutendes experimentelles Modell dar, um die Pathogenese und Behandlung von Hautveränderungen bei der caninen wie auch der humanen AD zu untersuchen.