

Pilot investigation of a model for canine atopic dermatitis: environmental house dust mite challenge of high-IgE-producing beagles, mite hypersensitive dogs with atopic dermatitis and normal dogs

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Abstract Although canine atopic dermatitis (cAD) is common, few models are available. The aim of this study was to evaluate high-IgE beagles epicutaneously sensitized to house dust mite (HDM) as a possible model for cAD. Six high-IgE beagles were environmentally challenged with HDM using various doses and protocols. Similar challenge protocols were used in positive and negative control dogs: three dogs with naturally occurring cAD and positive intradermal skin test (IDT) to HDM and three normal dogs without history of skin disease and negative IDT to HDM. All high-IgE beagles and all atopic dogs developed severe cutaneous lesions and pruritus after challenge. Lesions were erythematous papules and macules in contact areas such as face, ears, ventral abdomen, groin, axillae and feet. They were first visible after 6 h and increased in severity over time. No normal dog developed pruritus or lesions. Biopsies of representative lesions in the high-IgE beagles were taken for histopathology and immunohistochemistry. There was superficial perivascular dermatitis with mononuclear infiltrates and spongiosis. Lymphocytes and eosinophils accumulated in small epidermal micro-abscesses with hyperplasia of epidermal IgE-bearing dendritic cells. These findings suggest that this colony of high-IgE beagles develops a dermatitis that clinically, histopathologically and immunologically resembles the naturally occurring canine disease. It is also concluded that this modality of challenge is not irritating to normal dogs but induces flare-ups in hypersensitive atopic dogs.

INTRODUCTION

Atopic dermatitis (AD) is a chronic relapsing disease estimated to affect approximately 10% of the canine population.¹ Despite the fact that canine atopic dermatitis (cAD) is common, its pathogenesis has not been completely elucidated and limited treatment options are available for affected animals. AD has also become increasingly common in humans, affecting over 10% of children and constituting the most common cause of occupational disability in adults.^{2,3} Numerous similarities exist between cAD and its human counterpart,^{4,5} suggesting that the establishment of a model for cAD would be of benefit for both the canine and the human disease.⁶ More specifically, a model would enable in-depth evaluation of the pathogenesis of the disease

and rapid screening of specific treatments before undergoing lengthy clinical trials.

Numerous unsuccessful attempts have been made in the past to establish a model for cAD in dogs genetically predisposed to high IgE production.^{7,8} Unfortunately, the ability to induce high IgE responsiveness in those studies was not associated with the development of dermatitis after challenge. In one study, atopic dogs were bred to establish a colony of progeny predisposed to develop type I hypersensitivity.⁸ In a 2-year period, 72 pups were born and monitored for clinical evidence and immunologic 'markers' of atopic disease. Unfortunately, no correlation was found between the development of AD and the measurement of total and allergen-specific IgE and no indicators could be found to predict the development of the atopic state in those dogs.

In a more recent study, laboratory beagles genetically predisposed to produce high serum concentrations of allergen-specific IgE were used to establish a model for cAD.⁹ These dogs were subcutaneously sensitized from birth to several allergens, including house dust mite (HDM). At 15 months of age, they were continuously exposed to HDM. Despite the fact that these sensitized dogs had positive intradermal skin test (IDT) reactions and high serum allergen-specific IgE, only mild

Part of these studies was presented at the annual meeting of the 20th Meeting of the North American Veterinary Forum.

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transient erythema developed in a few dogs after challenge. The authors concluded that increases in concentrations of serum allergen-specific IgE and exposure to allergens was not sufficient to induce clinical signs of AD in genetically predisposed dogs.

Because of the complexity of atopic disease, it is reasonable to speculate that additional mutations are necessary to trigger AD besides high-IgE production. Therefore, the purpose of the present study was to evaluate another colony of high-IgE-producing beagles epicutaneously sensitized to HDM as possible model for AD. Investigation of this model was carried out by environmental challenge with HDM using various doses and protocols. Normal dogs with no previous history of skin disease and dogs with naturally occurring AD and are known to be allergic to HDM on IDT were similarly challenged and used as negative and positive controls, respectively.

MATERIALS AND METHODS

All procedures used in this study were approved by the institutional animal care use committee and by the clinical research review committee of the University of Florida as appropriate.

Environmental challenge of the high-IgE beagles (n = 6)

Six beagles epicutaneously sensitized to HDM at a young age and known to have positive atopy patch test (APT) reactions to HDM¹⁰ and positive IDT reactions at 15 min (25 protein nitrogen units mL⁻¹, mixture of *Dermatophagoides farinae* and *Dermatophagoides pteronyssus*, Greer Laboratories Inc, Lenoir, North Carolina, US) were used. Reactions to IDT with HDM were subjectively scored as 4+ in all dogs (0 was the score given to the saline injection and 4+ was the score given to the histamine used as positive control).

At the time of the experiments, the dogs were 5 years old. Five were intact females and one was an intact male. Dogs were housed in individual cement runs in a research facility of the University of Florida. Runs were cleaned daily using high-temperature and high-pressure wash. Stuffed toys, carpets, soft bedding or anything that could trap dust were not allowed in the runs. Walls of the runs and filters were checked monthly to ensure the absence of HDM in the environment in between challenges. Results were always below the detection level of the test used (MITE-T-Fast™ Allergen Detection System, Aveho Biosciences, Monterey, TN, USA).

In order to identify a model for naturally occurring disease, it was decided to challenge these dogs by environmental exposure to HDM. In the first set of experiments, the dogs were exposed to either *D. farinae* or saline (phosphate buffered saline, PBS) sprayed onto the bottom of a plastic air-crate. The HDM solution was prepared from a culture of HDM (natural *D. farinae*)

and stored at -20 °C until prepared for use. The investigator performing the clinical evaluations was blinded to the allocation of the dogs to the groups.

Once it was demonstrated that dermatitis could be elicited with environmental exposure to HDM, various protocols were used to identify the optimal dose and number of exposures required to trigger the development of dermatitis. Because of the low number of dogs and the previous demonstration of the development of dermatitis with HDM exposure, a negative control was not used in all the titration experiments. A 30-day minimum interval was observed in between exposures to allow dogs to recover from the experiments.

Exposure 1 (low-dose, 1-day exposure, HDM group n = 4, saline group n = 2). The first exposure used HDM at 0.31 mg mL⁻¹. A total volume of 1.6 mL was used, resulting in 0.5 mg per dog. This dose was selected based on previous studies (data not shown). The HDM was prepared on the day of treatment using filtered (0.2 µm syringe filter) PBS (pH = 7.2). The solution was vortexed and sonicated. The dogs were evaluated using a modified canine atopic dermatitis extent and severity index (mCADESI).¹¹ HDM solution (four dogs) or PBS (two dogs) was applied to the bottom of each dog's plastic air kennel by spraying it from the syringe. The kennel measured 50.8 × 68.6 × 48.3 cm. Each dog was placed in a kennel for 3 h and evaluated again at 24, 48, 72 and 96 h after the beginning of the exposure. In all experiments, kennels were not washed between exposures.

Exposure 2 (medium-dose, 3-day exposure, HDM group n = 4, saline group n = 2). This exposure was similar to exposure 1, executed over 3 consecutive days, with two control dogs. The concentration of HDM was increased to 3.1 mg mL⁻¹ in 1.6 mL, resulting in a dose of 5 mg per dog. The HDM or PBS was applied to the bottom of the dogs' kennels as described previously. All dogs remained in their kennels for 3 h each day. Dogs were evaluated before exposure and approximately 7 h after the start of each exposure. Evaluations continued every 24 h thereafter up to 96 h after the start of the first exposure or until the dog(s) required therapy for their symptoms. Biopsies were taken from two dogs (one saline dog and one HDM dog) prior to the first exposure and after exposure (at 24, 55, and 72 h), from representative lesions.

Exposure 3 (high-dose, 1-day exposure, HDM group n = 6). All six dogs were exposed to 50 mg of HDM (31 mg mL⁻¹ in 1.6 mL) for only 1 day (3-h exposure) and evaluated at 0, 6, 24, 48 and 72 h after the start of exposure. Biopsies were collected from three dogs after exposure to HDM (at 24, 48 and 72 h) from representative lesions.

Exposure 4 (high-dose, 3-day exposure, HDM group n = 4, saline group n = 2). This series of exposure

used HDM at 31 mg mL⁻¹ in 1.6 mL or 50 mg per dog, with two dogs serving as controls and receiving 1.6 mL of PBS. The protocol used for evaluations was the same as in exposure 2. Biopsies were collected from four dogs in the HDM group after exposure (at 31, 55 and 72 h) from representative lesions.

Assessment of repeatability of the challenge in IgE beagles (HDM group n = 6). In order to assess the repeatability of the results obtained with the exposures, all six dogs were challenged with the high dose (50 mg per dog), 3 days in a row in five separate experiments, approximately 3 months apart from each other. Dogs were evaluated as described in exposure 4.

Control dogs

Normal dogs (HDM group n = 3). As negative controls, three normal dogs (two research beagles and one privately owned dog of approximately the same size) with no previous history of skin disease and negative IDT to HDM at 15 min (25PNU mL⁻¹, Greer) were selected. Dogs were free of infections based on physical examination and cytology before the challenge. Dogs were challenged with exposure to HDM by the same protocol as for the high-IgE Beagles using the high dose (50 mg per dog), 3 days in a row and evaluated as previously described. Dogs were placed in plastic crates of the same size as the high-IgE beagles with HDM for 3 h, evaluated, and scored for cutaneous lesions and pruritus.

Dogs with naturally occurring AD (HDM group n = 3). Three privately owned dogs diagnosed with naturally occurring AD based on history, clinical signs and exclusion of other pruritic skin diseases were recruited from the clinics (Veterinary Medical Teaching Hospital, University of Florida). These dogs were determined to be sensitive to HDM based on IDT (reaction of 3 or higher at 15 min after ID injection of HDM at 25PNU mL⁻¹). All dogs were free of secondary infections prior to enrolment based on physical examination and cytology of lesional areas. These dogs were scheduled to be environmentally challenged with HDM (3 h per day, 3 days in a row). Clinical lesions and pruritus were scored daily. As these dogs were not the same size as the beagles (two were English bulldogs and one was a German shepherd), the HDM dose was calculated proportional to the dose used for the beagles. The two bulldogs were exposed in kennels that were 1.3 times the size of the beagle kennel. The German shepherd was exposed in a kennel that was two times the size of the beagle kennel. Although the concentration of HDM remained the same, the amount applied was adjusted accordingly, so that the bulldogs received 2.1 mL (66 mg per dog) and the German shepherd received 3.2 mL of 31.25 mg mL⁻¹ HDM (100 mg per dog) to account for the difference in size of the crate used.

Analysis of allergen content

Samples of the HDM were sent to Indoor Biotechnologies (Charlottesville, VA) for analysis of allergen content.

Clinical evaluations

All evaluations were based on an mCADESI.¹¹ Briefly, the dog's body was divided into small sections, each of which received a score based on the clinical signs evaluated. Total score was calculated by adding the scores of all clinical signs and body sites. Total score was used in the statistical analysis. Clinical signs evaluated included, but were not limited to, erythema (diffuse or unspecified), macules (erythematous or unspecified), papules, excoriations, alopecia, lichenification, scaling and pruritus. The scoring system ranged from 0 (absent) to 3 (severe) for each site and each symptom.

Biopsies

Biopsies were collected from the high-IgE beagles during exposure numbers 2–4 (no lesions were detected after exposure 1). Restrictions imposed by the institutional animal care and use committee on the number of biopsies allowed for each research dog in a lifetime limited the number of biopsies that could be harvested in these experiments. Biopsies were collected using 1% lidocaine and a disposable 8-mm biopsy punch (Miltex Instrument Company, Bethpage, NY). Samples were cut in half. One-half was placed in formalin and paraffin embedded. The second half was placed in OCT (optimal cutting temperature compound, Tissue-Tek, Sakura Finetek, Torrance, CA) and frozen in isopentane cooled to freezing in liquid nitrogen. The frozen samples were stored at -70 °C until shipped on dry ice for IHC. All biopsy sites were sutured routinely.

Histopathology and immunohistochemistry (IHC)

Five micrometer-sections of each skin biopsy were cut on a cryostat. Sections were air dried, fixed in acetone and stored at -80 °C until processing. Sections were stained using a three-step labelled streptavidin method as previously described.¹² Primary anticanine CD3, CD4, CD8, CD1c monoclonal antibodies were gifts from Dr Moore (University of California, Davis, CA). The anticanine IgE was obtained from Dr Hammerberg (North Carolina State University, Raleigh, NC, USA) and used as previously described.¹³

STATISTICS

Clinical scores were analysed using least squares analysis of variance (Lsanova) with all main effects and interactions included in the model. Differences among doses were analysed using orthogonal contrast analysis.

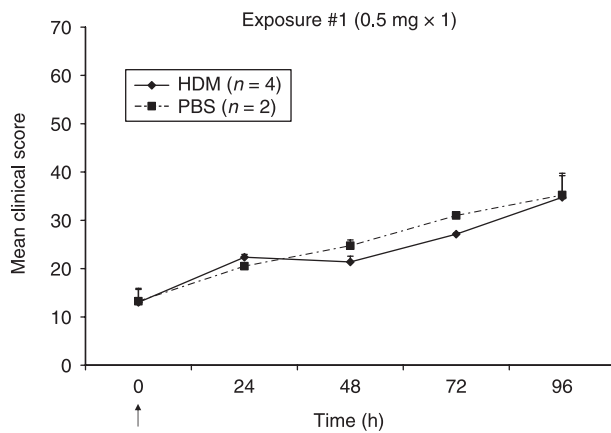


Figure 1. Mean clinical scores after environmental exposure to low dose of HDM (0.5 mg per dog), one-time, and 3-h exposure. The six dogs were divided into two groups: two dogs were exposed to saline and four dogs were exposed to HDM. The arrow indicates the time when the dogs were challenged. Clinical scores were not significantly different between the two groups at any time point.

Because clinical scores are not normally distributed, they were rank transformed prior to analysis. Means and standard error of the mean (SEM) were calculated using the untransformed data. However, all *P* values are representative of the rank-transformed data. In addition, tests for heterogeneity of regression were also conducted to evaluate time trends between treatments. The analysis was performed at the highest significant order of regression.

For the experiments evaluating the repeatability of the exposures in the high-IgE beagles, clinical scores were analysed using repeated-measures ANOVAs with both exposure and time of evaluation as within-subject factors. When planned comparisons are reported, exposure differences at each evaluation time were analysed using one-way ANOVAs with multiple levels of exposure.

All analyses were performed using the statistical software package The SAS system for Windows version 9.0 (SAS Institute, Cary, NC). A *P* < 0.05 was considered significant. All data are presented as mean ± SEM, unless otherwise indicated.

RESULTS

Environmental challenge of the high-IgE beagles

Exposure 1 (low-dose, 1-day exposure, HDM group n = 4, saline group n = 2).

Mean scores for the four dogs exposed to HDM and the two dogs exposed to saline increased over time (Fig. 1). Over all times, there was no statistically significant difference between the groups (*P* = 0.64). At each time, there were no statistically significant differences between the groups (*P* > 0.05). Within the HDM-exposed group, scores were significantly greater than baseline (0 h) from 24 to 96 h post-exposure. Within the PBS-exposed group, scores were significantly greater than baseline (0 h) from 48 to 96 h post-exposure.

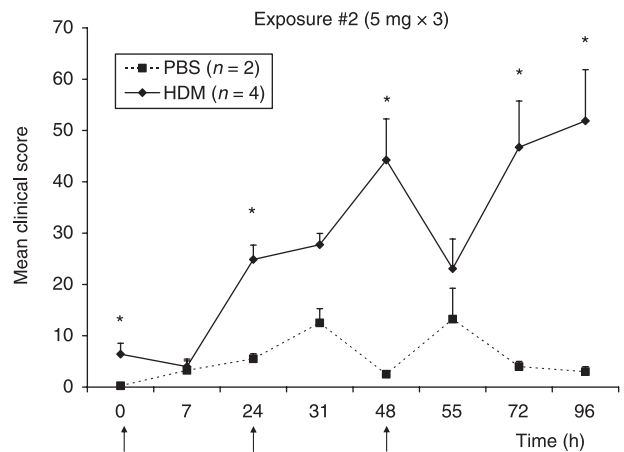


Figure 2. Mean clinical scores after medium dose of HDM (5 mg per dog). Dogs were exposed three times, 3 days in a row, 3-h exposure each time (exposure 2). The six dogs were divided into two groups: two dogs were exposed to saline and four dogs were exposed to HDM. The arrows indicate the times of challenge. The two groups were statistically significantly different and the asterisks indicate the times at which the difference was statistically significant.

Exposure 2 (medium-dose, 3-day exposure, HDM group n = 4, saline group n = 2)

Clinical scores. Mean scores for the four dogs exposed to HDM and the two dogs exposed to saline varied over time, but the HDM group scores continued to increase over time, whereas the saline group decreased towards the end of the experiment (Fig. 2). A statistically significant difference between the groups (*P* = 0.02) over all times was found. The groups were significantly different at 0, 24, 31, 48, 72 and 96 h (*P* ≤ 0.01). Within the HDM-exposed group, scores were significantly greater than baseline (0 h) from 7 to 48 h and 72–96 h post-exposure (*P* ≤ 0.002). At 55 h post-exposure, the HDM-exposed scores were almost significantly different (*P* = 0.05) from baseline (0 h). Within the PBS-exposed group, scores were significantly greater than baseline (0 h) at 24, 31 and 55 h post-exposure. One dog in the HDM group was withdrawn after the 72-h evaluation because of the severity of skin lesions and pruritus. Systemic glucocorticoids and topical therapy with a leave-on hydrocortisone conditioner were prescribed to resolve the lesions. No antibiotics were required for the resolution of the lesions.

Histopathology. Biopsies were collected from one dog in the HDM group and from one in the saline group. One biopsy from each dog was taken prior to the exposure from the inguinal area. After exposure, two biopsies were collected from representative lesions at 24, 55 and 72 h from each of the dogs.

Prior to the challenge (0 h) the biopsies of all dogs had no significant findings on histopathology. On IHC, rare CD3⁺ cells were seen in the superficial dermis. Hyperplastic dendritic Langerhans cells (LC) were detected. Occasional dermal dendritic cells were seen throughout the dermis with rare foci of aggregation.

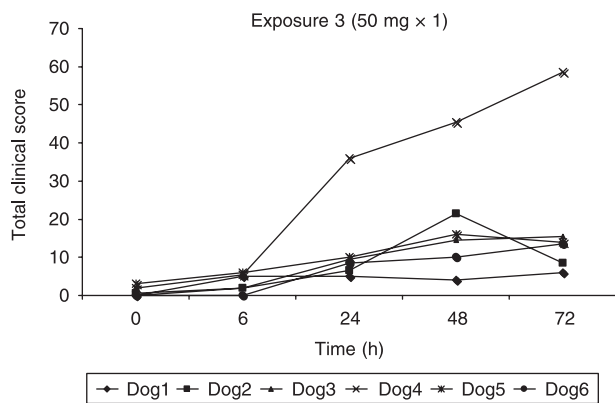


Figure 3. Total clinical scores of the individual dogs exposed to the high dose (50 mg per dog), one 3-h exposure (exposure 3). In this experiment, all the dogs were exposed to HDM and there were no controls. The arrow indicates the time when the dogs were challenged. Note that the scores started to increase after 24 h and in 5/6 dogs reached a peak at 48 h, whereas in one dog, the score increased throughout the experiment.

IgE was highly expressed on mast cells. Low expression of IgE was detected on dermal dendritic cells (DDC).

In the dog exposed to HDM, lesions macroscopically classified as papules did not have significant histological changes at 24 h. However, on IHC, a mild mononuclear infiltrate was detected. Rare superficial dermal dendritic cells were noted and IgE was highly expressed on mast cells. In the same dog, papule biopsies at 55 h revealed multifocal epidermal hyperplasia, focal parakeratosis, focal intracorneal pustules and superficial perivascular dermatitis. The infiltrate was mixed, composed of both mononuclear cells and mast cells. Occasional dermal, often degranulated, eosinophils were detected (Luna stain). CD1c⁺ hyperplastic LC and DDC were present. Mononuclear cells were CD3⁺. IgE was highly expressed on both mast cells and DDC, whereas it was rarely expressed on LC. At 72 h, mild, diffuse, epidermal hyperplasia, subcorneal pustules (neutrophilic and eosinophilic), dermal oedema, superficial perivascular to diffuse dermatitis composed of mononuclear cells with rare eosinophils and eosinophilic exocytosis were present. Luna stain revealed rare dermal and epidermal, often degranulated, eosinophils. CD1c⁺ hyperplastic clusters of LC were detected in the epidermis and numerous hyperplastic DDC with clustering were seen in the dermis. Mononuclear cells were all CD3⁺. IgE was highly expressed on mast cells, and DDC. IgE was also detected on low affinity receptors on DDC.

Biopsies from the dog exposed to PBS did not have significant histopathological and immunohistochemical findings at any time point except high expression of IgE on mast cells.

Exposure 3 (high-dose, 50 mg × 1-day, HDM group n = 6)

Clinical scores. Pruritus and erythema were noted in all dogs. Clinical scores increased over time being significantly higher than baseline beginning at 24 h

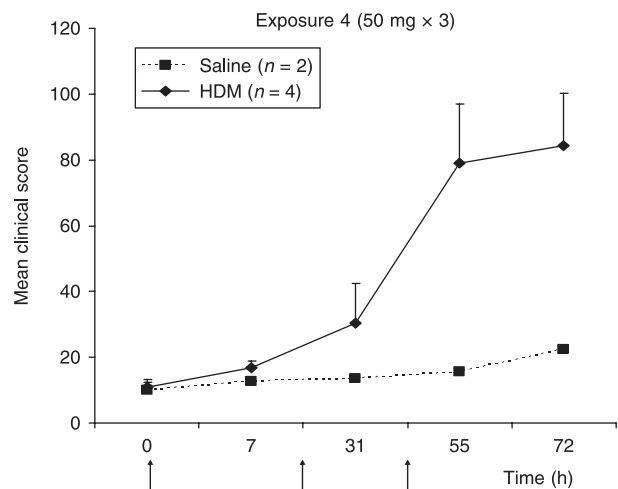


Figure 4. Mean clinical scores after the high dose (50 mg per dog), 3-day exposure (exposure 4). The six dogs were divided into two groups: two dogs were exposed to saline and four dogs were exposed to HDM. The arrows indicate the times of challenge. The asterisk indicates the time at which the two groups were different. Note that the scores of the dogs challenged with HDM progressively increased over the course of the experiment.

(Fig. 3). One dog required systemic glucocorticoids, but lesions in the other dogs resolved with topical hydrocortisone therapy.

Histopathology. Biopsies were collected from representative lesions in one dog at 24 h and in two dogs at 48 and 72 h after challenge with HDM. The biopsy of a papule at 24 h revealed mild superficial oedema with focal superficial dermal/junctional and epidermal neutrophilic and mononuclear aggregates. Papules biopsied at 48 h revealed superficial perivascular neutrophilic dermatitis and mononuclear dermatitis with rare eosinophils. Superficial epidermal (subcorneal) neutrophilic pustules and oedema were also noted at 48 h. Biopsies of papular lesions at 72 h revealed superficial perivascular mononuclear and neutrophilic dermatitis with superficial dermal oedema. Focal small neutrophilic pustules were also present. Bacteria were not detected.

Exposure 4 (high-dose, 50 mg × 3 days, HDM group n = 4, saline group n = 2)

Clinical scores. Mean scores for both groups increased over time, although the HDM group scores increased more than the saline group starting at 31 h after the beginning of the challenge (Fig. 4). Over all times, there was no significant difference between the groups ($P = 0.63$). At 55 h, the score of the HDM group was significantly higher ($P = 0.02$) than the score of the PBS group. Within the HDM-exposed group, scores were significantly greater than baseline (0 h) from 7 to 72 h post-exposure. Within the PBS-exposed group, scores were only significantly greater than baseline (0 h) at one time (72 h). Because of the severity of the reactions in the HDM group, the experiment was ended prematurely at 72 h.



Figure 5. Primary papular eruption on the front legs of one of the high-IgE beagles challenged with HDM (50 mg). At the time of the picture, the dog had been challenged twice, at 0 and 24 h. Each challenge consisted of a 3-h exposure to HDM. Picture was taken 48 h after the beginning of the first challenge.



Figure 6. Severe erythema and pruritus on the muzzle of one of the high-IgE beagles challenged with HDM (50 mg, 3 h per challenge, and 3 days in a row). The picture was taken 48 h after the beginning of the first challenge. Erythema had progressively increased over time and was severe after the third challenge. Because of the severity of the pruritus, this dog was treated with systemic glucocorticoids at the end of the challenge.

All four dogs exposed to HDM developed clinical lesions as consequence of the challenge. Three of the four dogs exposed to HDM developed an erythematous maculopapular dermatitis that started on the ventral abdomen as early as the second exposure. Lesional scores in the HDM group increased up to sevenfold. In the dogs challenged with HDM, small papules were also noted on the front legs (Fig. 5). Erythema, pruritus and papular eruptions progressively increased over time and became severe at 55 h. The distribution of lesions was similar to naturally occurring CAD and included ventral abdomen, axillae, groin, pinnae and face (Fig. 6). One dog required systemic glucocorticoids, but lesions in all other dogs resolved with topical glucocorticoid therapy. No antibiotics were necessary.

Histopathology. Biopsies were collected on representative lesions only in the group exposed to HDM. These included one dog at 31 h, two at 55 h, and one at 72 h.

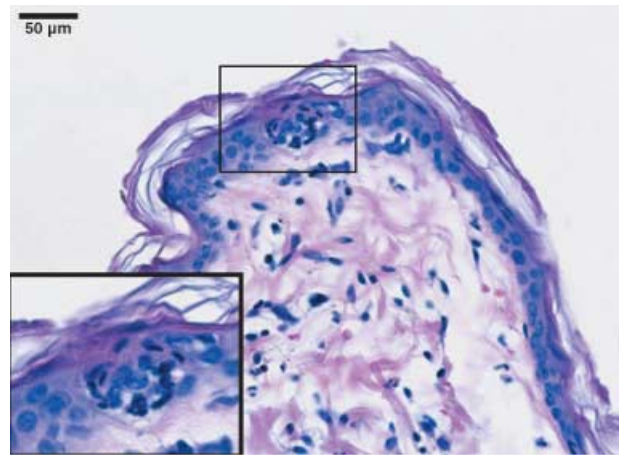


Figure 7. Mononuclear cells microaggregates developed in the epidermis of the high-IgE beagles upon challenge with HDM (50 mg, 3 h per challenge, 3 days in a row). Biopsy was taken 55 h after the beginning of first challenge. Bar = 50 µm, inset = 25 µm.

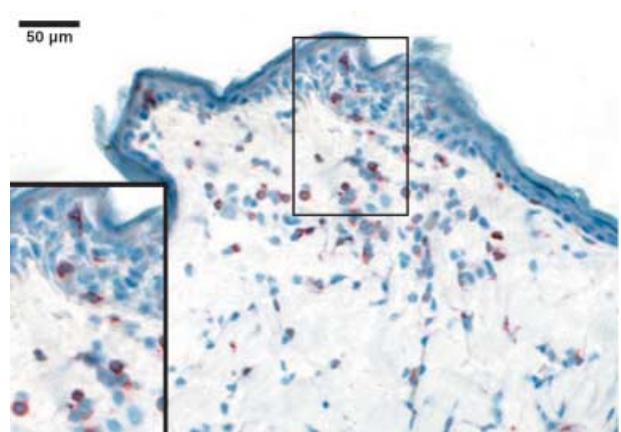


Figure 7a. Immunohistochemistry on the same slide indicated that many mononuclear cells infiltrating the epidermis stained positive for CD3⁺ and were therefore identified as T lymphocytes. Bar = 50 µm; inset: 33 µm.

Biopsies from erythematous macules at 31 h revealed superficial perivascular mononuclear dermatitis with oedema. By 55 h, CD3⁺ mononuclear cells were aggregating in the epidermis (Figs 7, 7a). Eosinophils were activated and degranulating in the dermis (Luna stain). Epidermal eosinophil exocytosis, abscess formation and spongiosis were seen (Fig. 8). Langerhans cells were seen in close association with the eosinophils abscesses. Mast cells and dendritic cells exhibited surface IgE at high levels (Fig. 9). Clusters of CD1c⁺ cells were present in the centre of the papular lesions (Fig. 10), suggesting a role of dendritic cells in the epicutaneous capture of the allergen. CD4⁺ and CD8⁺ cells were infiltrating the epidermis and dermis. Both the lymphocytes and dendritic cells expressed CD4. Papular lesions at 72 h revealed degranulating eosinophils and a hyperplastic and spongiotic superficial to diffuse dermatitis with subcorneal microabscesses.

Assessment of repeatability of the challenge in IgE beagles (HDM group n = 6). When all six high-IgE

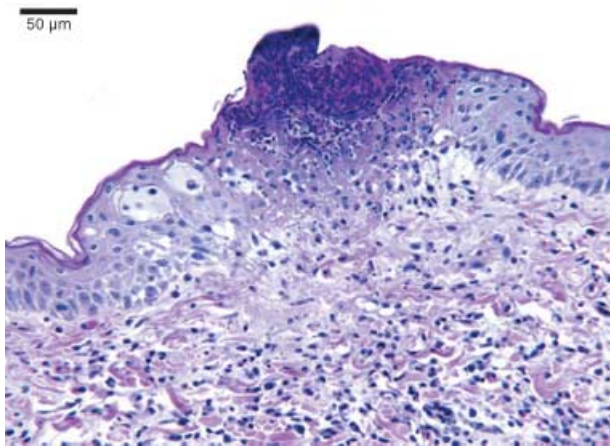


Figure 8. Spongiosis, eosinophilic exocytosis and microabscess formation in the centre of a papular lesion biopsied 55 h after the beginning of first challenge with HDM (50 mg, 3 h per challenge, 3 days in a row) in a high-IgE beagle. Bar = 50 µm.

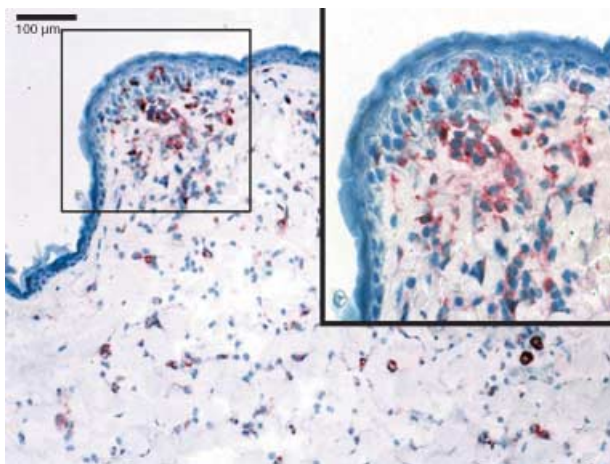


Figure 9. Immunohistochemical stain for IgE. This biopsy was taken 55 h after the beginning of the challenge with HDM (50 mg, 3 h per challenge, 3 days in a row) in a high-IgE Beagle. Both mast cells and dendritic cells exhibit surface IgE. Note the dendritic cells infiltrating the epidermis and the mast cells in the dermis. Bar = 100 µm; inset: 60 µm.

beagles were challenged with the high-dose protocol (50 mg per dog, 3 days in a row) on five different challenges, every dog developed lesions and pruritus in each challenge. When the scores of the challenges were compared, it was found that there was a difference between challenges, with the first exposure triggering the highest scores ($P = 0.045$, Fig. 11). In addition, the exposures differed as a function of time (time-exposure interaction, $P < 0.01$). One-way ANOVAS at each time point revealed that exposures were different at 24, 48 and 96 h ($P = 0.029$, 0.019 and 0.019, respectively). Importantly, in all exposures there was a significant increase of scores after challenges compared to the baseline starting at 48 h ($P < 0.001$).

Control dogs: normal dogs (n = 3) and dogs with naturally occurring AD (n = 3)

Research dogs (two normal beagle dogs). None of the

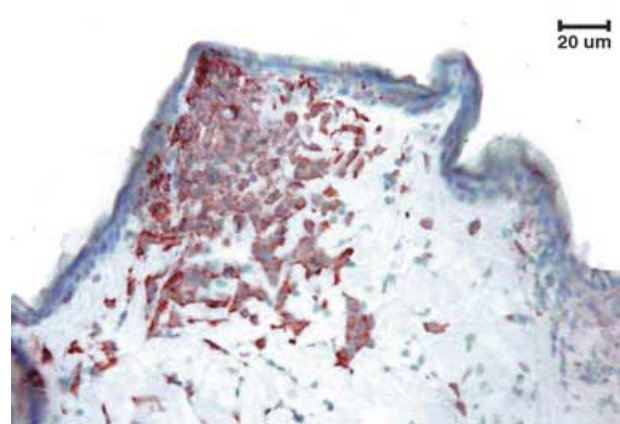


Figure 10. Immunohistochemical stain for CD1c. This biopsy was taken 55 h after the beginning of the challenge with HDM (50 mg, 3 h per challenge, 3 days in a row) in a high-IgE beagle and demonstrates CD1c⁺ cells accumulating in clusters in the centre of papular lesions. These findings are similar to those of the naturally occurring disease and suggest a role of dendritic cells in the epicutaneous capture of the allergen. Bar = 20 µm.

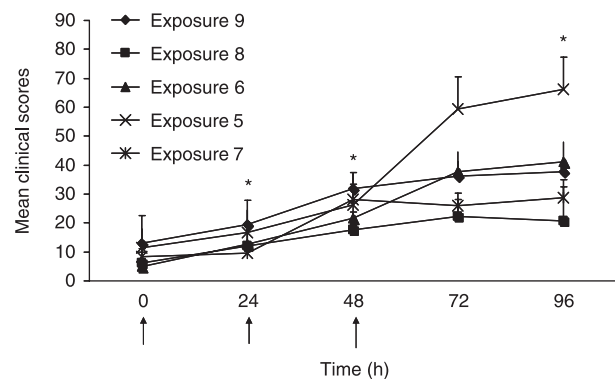


Figure 11. Mean clinical scores of five exposures of the six high-IgE beagles using high dose (50 mg per dog), 3 h per challenge, 3 days in a row. Exposures were performed approximately 3 months apart from each other. The arrows indicate the times when challenges were carried out. Asterisks indicate times at which significant difference were detected between challenges.

normal beagles developed lesions upon environmental challenge (high dose, 50 mg $\times$ 3 days). Scores did not change over time (Fig. 12).

Privately owned dogs (one normal and three atopic). The privately owned dogs were scheduled to be challenged with the same protocol used for the research beagles (high dose, 3, days in a row). However, after the first exposure, the dogs with natural AD developed such severe lesions and pruritus that the normal dogs were not further exposed. For consistency of the experiment and to prevent possible exposure of the cAD dogs to additional HDM, the normal dog was also exposed only once. The dogs remained housed in the same room but were not exposed to additional HDM. Evaluation was made at 0, 6, 24, 30, and 48 h after the start of the first exposure. With only 1 normal dog, the results of the clinical evaluations were not compared statistically. A graphic representation of the scores is presented in succeeding discussions (Fig. 13).

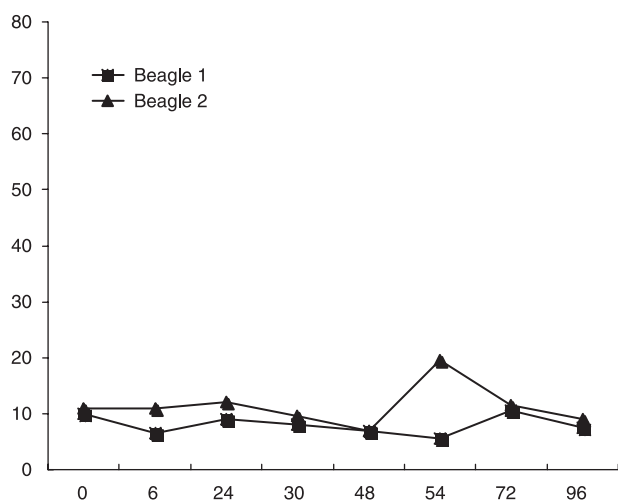


Figure 12. Total scores of two normal research beagles challenged with the high-dose protocol (50 mg per dog, 3 days in a row, and 3 h per challenge). These dogs had been negative on a previous intradermal skin tested for HDM. None of the dogs developed lesions or pruritus.

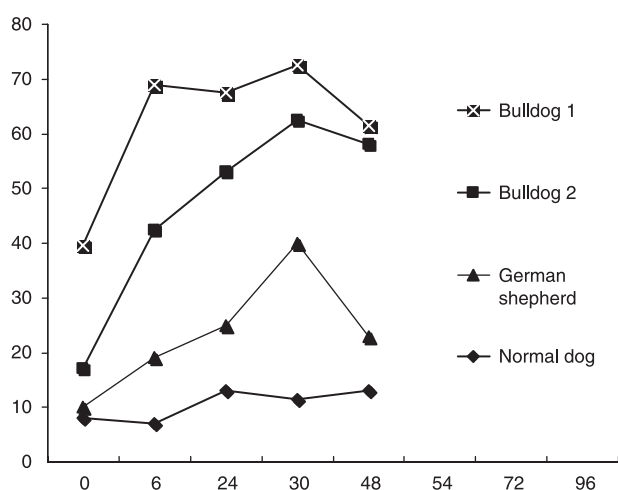


Figure 13. Total scores of three privately owned dogs (one normal dog and three dogs with spontaneous AD) challenged once with the 50-mg HDM, 3-h exposure. Experiment was ended prematurely at 48 h because of severe reactions of the AD dogs. Because of the low numbers, no statistics were performed.

All of the atopic dogs developed lesions and pruritus after challenge, whereas the normal dogs did not. Two (bulldogs) out of three AD dogs developed erythema and macular papular eruption on the face, feet, ears, ventral abdomen, and intense pruritus starting 6 h after exposure (Figs 14, 14a, 15 and 15a). Severe erythema, facial pruritus and conjunctivitis developed at 24 h. The third dog (German shepherd) also developed erythema and pruritus but of less intensity than that in the bulldogs. After 48 h, it was deemed necessary to withdraw the dogs from the study because of the severity of inflammation and pruritus. The dogs were bathed and started on oral prednisone therapy.



Figure 14. Ear pinna of one of the atopic dogs (bulldog) before exposure to HDM (50 mg, one exposure). This dog was selected because of positive intradermal skin test to HDM.



Figure 14a. Same dog 6 h after a single challenge with HDM (50 mg). Note the intense erythema. Severe pruritus was present.

Analysis of allergen content

Results of the HDM analysis revealed that 31 mg mL⁻¹ contained an average (mean±SD) of 183 ± 14.1 µg mL⁻¹ of Derf1, 0.34 ± 0.02 µg mL⁻¹ of Derp1. Mite group 1 content was 183.34 ± 14.1 µg mL⁻¹ and mite group 2 was 1.975 ± 0.3 µg mL⁻¹.

DISCUSSION

In this study, the development of a dermatitis that clinically, histologically and immunologically resembles cAD is described in a colony of high-IgE beagles. The dogs used in the experiment were epicutaneously sensitized to HDM in the first few weeks of life and shown to develop positive APT with HDM.¹⁰ The present study further developed the model by eliciting dermatitis



Figure 15. Interdigital space of one of the atopic dogs (bulldog) before exposure to HDM (50 mg, one exposure). This dog was selected because of positive intradermal skin test for HDM.



Figure 15a. Same dog 6 h after challenge with HDM. Note the severe erythema. Intense pruritus was also present.

after environmental exposure to HDM. The ability to obtain both a positive APT and generalized dermatitis and pruritus upon environmental challenge in all dogs makes this colony unique.

Experimental sensitization in dogs has revealed that the capacity to produce high levels of IgE against allergens (high-IgE responders) is a genetic trait inherited in a dominant manner.¹⁵ In high-IgE responder dogs, spontaneous development of IgE to inhaled allergens, such as HDM, represents an apparent phenotype very similar to that observed in human atopic families.¹⁶ The full potential of the high-IgE-response gene appears to be fulfilled only under some conditions such as early and repeated exposure to allergens. Differences in animal selection, sensitization method and experimental protocol may explain the difference in results between studies. In a recent study by Egli and coworkers,

sensitization of high-IgE producing beagles at an early age failed to reproduce clinical AD upon challenge, despite the fact that these dogs had elevated levels of allergen-specific IgE.⁹ The dogs were sensitized by subcutaneous injections, bypassing the skin barrier and the interaction between the allergen and epidermal LC. The dogs used in our study were sensitized epicutaneously in an attempt to mimic the naturally occurring disease. Therefore, the sensitization method might have affected the ability of the dogs to react to epicutaneous exposure of allergen later in life. This hypothesis is also supported by the fact that in Egli's study no IgE+ cells were found in the epidermis, whereas in the present study, IgE+ dendritic cells were found to accumulate in the lesions, confirming a role for IgE and dendritic cells in allergen capture. These findings are similar to reports in human^{17,18} and cAD,¹⁹ suggesting that the dermatitis observed in our dogs closely mimics spontaneous disease.

Another factor that may affect results is the source and dose of HDM. In Egli's study, 2 µg of mite allergen per gram of dust were used. This was applied twice weekly on the carpet. The authors did not specify whether the dust was removed from the carpet in between exposures, how much total dust was used for each exposure and which HDM allergen was measured; therefore a direct comparison between methodologies is not possible.

In the present study, HDM in liquid form rather than dust was used for the environmental challenges so the concentrations are expressed in milligram per millilitre. For example, in the most concentrated challenge, 1.6 mL of 31 mg mL⁻¹ HDM was applied, for a total amount of 50 mg per dog for a 3-h environmental challenge. It is interesting to note that although the HDM used for these challenges was *D. farinae*, minor quantities of *D. pteronyssus* allergens were detected in the analysis. This could either be resulting from the fact the culture was not 100% pure or that some cross-reactivity between allergens was present in the assay used. The HDM product used for the high-dose challenges revealed 183 ± 14.1 µg mL⁻¹ of Derf1, 0.34 ± 0.02 µg mL⁻¹ of Derp1. Average mite group 1 content was 183.34 ± 14.1 µg mL⁻¹ and mite group 2 was 1.975 ± 0.3 µg mL⁻¹.

A relevant question might then be how the amount of HDM used in these experiments relates to that present in the environment. A direct comparison is once again difficult, as most reports in the literature refer to the amount of allergen per gram of dust. In addition, the quantity of mites in the environment varies, depending on temperature and humidity with more mites in tropical climates. One study of dust mites in Singapore found more than 500 mites per gram in all samples from sofas and carpets.²⁰ Another study measuring HDM and HDM allergens in the microenvironment of dogs in homes in Ohio found Derf in all homes.²¹ The mean level of Derf1 was 2.25 ± 4.97 µg g⁻¹. The mean level of Derp1 was 1.8 ± 5.36 µg g⁻¹. Houses associated with the highest levels had a relative

humidity higher than 75%. The total amount of dust present in the environment was not reported. In a European study, mattresses of people not allergic to mites contained an average of 287.9 µg of mite group 1 per gram of dust, whereas in houses where people were reportedly allergic to dust and implemented measures to reduce dust, the mean level decreased to 190 µg g⁻¹.²² Based on these results and assuming 1 gram of dust to be equivalent to 1 mL of the liquid used for the challenges in the present experiments, the content of mites used in the high-dose experiments is similar to that found in mattresses (e.g. average mite group 1 content of HDM used was 183.34 mg mL⁻¹).

To explain differences between our study and Egli's, it is also important to consider the possibility that the ability to induce AD lesions may not be entirely dependent on the ability to produce IgE, and that additional mutations leading to the development of lesions may be present in the colony used in this study. It is becoming increasingly evident that IgE antibodies do not completely explain the pathogenesis of AD and that additional mechanisms must be involved in determining clinical disease. *D. farinae*-specific IgE is commonly found in clinically normal dogs, and this is usually associated with a positive IDT reaction.²³ It has been suggested that it is the amount of IgE that determines which dog develops disease.²³ However, it has been demonstrated that total serum IgE concentrations in healthy dogs, parasitized dogs and atopic dogs do not differ from each other significantly.²⁴ Alternatively, IgE may be functionally heterogeneous and additional factors influencing mediator release could be involved in the development of AD.^{25,26} In humans, approximately 20% of patients with clinically diagnosed AD do not have increased allergen-specific IgE and positive IDT.^{27,28} This form of the disease has been called intrinsic or nonallergic AD.²⁹ It is possible that, in dogs too, IgE may just be an epiphenomena and not strictly necessary to cause disease. In the present study, the dogs were selected because they were high-IgE responders. However, it is reasonable to speculate that some additional mutation may exist in this colony to explain why these dogs developed disease when previous attempts had failed. A variety of gene candidates and mutations have been evaluated in human AD.^{30–33} Further studies will be necessary to evaluate possible genetic mutations present in this colony of dogs.

In the current model, all IgE beagles developed pruritus and primary lesions consisting of erythematous macules and extremely small papules. The clinical response appeared to be dose-dependent and related to the number of exposures. Clinical lesions could be elicited in these dogs each time they were challenged with the high-dose protocol, although some variation in the severity of scores was found between exposures. Histological examination of papular lesions revealed accumulations of LC (suggestive of local capture of the allergen via IgE) and eosinophils in the epidermis. The presence of IgE-bearing LCs in primary lesions confirms that these reactions were indeed allergic and not

irritant in nature. Furthermore, when normal dogs were challenged with the same concentration of HDM, no primary lesions were observed, whereas dogs with naturally occurring AD, known to be HDM-sensitive, flared after only one exposure.

It is important to note that some worsening of scores was also observed in the placebo-exposed high-IgE beagles. This may partly be the result of housing of all dogs in the same room, although they were exposed in separate kennels. Therefore, it is conceivable that some inhalation of HDM may have occurred in the dogs allocated to the saline group.

According to Willemse's suggested guidelines for the diagnosis of canine AD, at least three major and three minor criteria should be fulfilled.³⁴ These criteria represent an adaptation of the Hanifin's criteria used in human medicine.^{35,36} Major diagnostic criteria for canine AD include pruritus, facial or digital involvement, involvement of the flexor and extensor surface of the joints, chronic or chronically relapsing dermatitis, an individual or family history of atopy and a breed predilection. The minor criteria include onset of signs before 3 years of age, facial erythema and cheilitis, bilateral follicular conjunctivitis, superficial staphylococcal pyoderma, xerosis, hyperhydrosis, immediate skin test reactivity, high allergen-specific IgG and/or IgE. The high-IgE dogs used in this study fulfilled most of the criteria listed, as they all developed pruritus, facial and/or digital involvement, and involvement of the flexor and extensor surface of the joints. Some also developed conjunctivitis and cheilitis. All these dogs were genetically related, had high IgE to HDM and positive IDT and APT.

To further evaluate whether the cutaneous lesions observed in the high-IgE beagles represented AD, biopsies for histopathology and IHC were taken. The histopathological findings in our model are identical to those reported in animals with naturally occurring disease, confirming that these dogs represent a suitable model for cAD,^{12,19} with IgE+ dendritic cells in the epidermis similar to that reported in humans with AD.³⁷

Limitations of this study included the small number of dogs in the colony and even smaller number of privately owned dogs used for the exposures. Once the flare-up of dermatitis was demonstrated in privately owned AD dogs, ethical considerations prevented the challenge of additional dogs. Although statistics were either not carried out (as in the privately owned dogs) or may have limited power (high-IgE beagles), these experiments were intended as a proof of concept and, in that light, are considered important. Institutional animal care and use committee also limited the number of biopsies that were allowed in each high-IgE beagle. As these dogs were planned for use in other experiments, not all dogs were biopsied at all times. The high-IgE beagles are currently being bred to allow the expansion of the colony and the retirement of the founders.

In conclusion, the results of this study identify a colony of high-IgE-producing beagles that after envi-

ronmental allergen challenge, develop a dermatitis that is clinically, histologically and immunologically similar to naturally occurring cAD. This model could be used in the future to better elucidate the pathogenetic mechanisms involved in cAD and allows rapid screening of new therapeutic options.

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REFERENCES

- Hillier A, Griffin CE. The ACVD task force on canine atopic dermatitis (I): incidence and prevalence. *Veterinary Immunology and Immunopathology* 2001; 81: 147–52.
- Feijen M, Gerritsen J, Postma DS. Genetics of allergic disease. *British Medical Bulletin* 2000; 56: 894–907.
- Williams HC. Epidemiology of atopic dermatitis. *Clinical and Experimental Dermatology* 2000; 25: 522–9.
- Willemse T. Comparative aspects of canine and human atopic dermatitis. *Seminars in Veterinary Medicine and Surgery (Small Animal)* 1988; 3: 255.
- Helton Rhodes K, Kerdel F, Soter NA. Comparative aspects of canine and human atopic dermatitis. *Seminars in Veterinary Medicine and Surgery (Small Animal)* 1987; 2: 166–72.
- Marsella R, Olivry T. Animal models of atopic dermatitis. *Clinics in Dermatology* 2003; 21: 122–33.
- Schwartzman RM, Massicot JG, Sogn DD *et al.* The atopic dog model: report of an attempt to establish a colony. *International Archives of Allergy and Applied Immunology* 1983; 72: 97–101.
- Schwartzman RM. Immunologic studies of progeny of atopic dogs. *American Journal of Veterinary Research* 1984; 45: 375–8.
- Egli KS, Schiessl B, Roosje PJ *et al.* Evaluation of the usefulness of sensitization to aeroallergens as a model for canine atopic dermatitis in genetically predisposed Beagles. *American Journal of Veterinary Research* 2002; 63: 1329–36.
- Olivry T, Buckler KE, Dunston SM *et al.* Positive 'atopy patch tests' reactions in IgE-hyperresponsive beagle dogs are dependent upon elevated allergen-specific IgE serum levels and are associated with IgE-expressing dendritic cells. *Veterinary Dermatology* 2002; 13: 219.
- Olivry T, Rivierre C, Jackson HA *et al.* Cyclosporine decreases skin lesions and pruritus in dogs with atopic dermatitis: a blinded randomized prednisolone-controlled trial. *Veterinary Dermatology* 2002; 13: 77–87.
- Olivry T, Naydan DK, Moore PF. Characterization of the cutaneous inflammatory infiltrate in canine atopic dermatitis. *American Journal of Dermatopathology* 1997; 19: 477–86.
- Gebhard D, Orton S, Edmiston D *et al.* Canine IgE monoclonal antibody specific for a filarial antigen: production by a canine $\times$ murine heterohybridoma using B cells from a clinically affected lymph node. *Immunology* 1995; 85: 429–34.
- Butler JM, Peters JE, Hirshman CA *et al.* Pruritic dermatitis in asthmatic basenji-greyhound dogs: a model for human atopic dermatitis. *Journal of the American Academy of Dermatology* 1983; 8: 33–8.
- Denburg JA, Inman MD, Wood L *et al.* Bone marrow progenitors in allergic airways diseases: studies in canine and human models. *International Archives of Allergy and Immunology* 1997; 113: 181–3.
- de Weck AL, Mayer P, Stumper B *et al.* Dog allergy, a model for allergy genetics. *International Archives of Allergy and Immunology* 1997; 113: 55–7.
- Semper AE, Heron K, Woollard AC *et al.* Surface expression of Fc epsilon RI on Langerhans cells of clinically uninvolved skin is associated with disease activity in atopic dermatitis, allergic asthma, and rhinitis. *Journal of Allergy and Clinical Immunology* 2003; 112: 411–9.
- Schuller E, Teichmann B, Haberstock J *et al.* In situ expression of the costimulatory molecules CD80 and CD86 on langerhans cells and inflammatory dendritic epidermal cells (IDEC) in atopic dermatitis. *Archives of Dermatological Research* 2001; 293: 448–54.
- Olivry T, Moore PF, Affolter VK *et al.* Langerhans cell hyperplasia and IgE expression in canine atopic dermatitis. *Archives of Dermatological Research* 1996; 288: 579–85.
- Chew FT, Zhang L, Ho TM *et al.* House dust mite fauna of tropical Singapore. *Clinical and Experimental Allergy* 1999; 29: 201–6.
- Randall AR, Hillier A, Kwotchka KW *et al.* Quantification of house dust mites and house dust mite allergens in the microenvironment of dogs. *American Journal of Veterinary Research* 2003; 64: 1301–9.
- Hirsch T, Range U, Walther KU *et al.* Prevalence and determinants of house dust mite allergen in East German homes. *Clinical and Experimental Allergy* 1998; 28: 956–564.
- Halliwell RE, Gilbert SM, Lian TM. Induced and spontaneous IgE antibodies to *Dermatophagoides farinae* in dogs and cats: evidence of functional heterogeneity. *Veterinary Dermatology* 1998; 9: 179–84.
- Hill PB, Moriello KA, DeBoer DJ. Concentrations of total serum IgE, IgA and IgG in atopic, normal and parasitized dogs. *Veterinary Immunology and Immunopathology* 1995; 44: 105–13.
- Jackson HA, Miller HR, Halliwell RE. Canine leucocyte histamine release: response to antigen and to anti-IgE. *Veterinary Immunology and Immunopathology* 1996; 53: 195–206.
- Peters JE, Hirshman CA, Malley A. The Basenji-Greyhound dog model of asthma: leukocyte histamine release, serum IgE, and airway response to inhaled antigen. *Journal of Immunology* 1982; 129: 1245–9.
- Novak N, Bieber T. Allergic and nonallergic forms of atopic diseases. *Journal of Allergy and Clinical Immunology* 2003; 112: 252–62.
- Wuthrich B, Schmid-Grendelmeier P. The atopic eczema/dermatitis syndrome. Epidemiology, natural course, and immunology of the IgE-associated ('extrinsic') and the nonallergic ('intrinsic') AEDS. *Journal of Investigational Allergology and Clinical Immunology* 2003; 13: 1–5.
- Akdis CA, Akdis M. Immunological differences between intrinsic and extrinsic types of atopic dermatitis. *Clinical and Experimental Allergy* 2003; 33: 1618–21.
- Nishio Y, Noguchi E, Ito S *et al.* Mutation and association analysis of the interferon regulatory factor 2 gene (IRF2) with atopic dermatitis. *Journal of Human Genetics* 2001; 46: 664–7.

31. Soderhall C, Bradley M, Kockum I et al. Linkage and association to candidate regions in Swedish atopic dermatitis families. *Human Genetics* 2001; 109: 129–35.
32. Oiso N, Fukai K, Ishii M. Interleukin 4 receptor alpha chain polymorphism Gln551Arg is associated with adult atopic dermatitis in Japan. *British Journal of Dermatology* 2000; 142: 1003–6.
33. Nickel RG, Casolaro V, Wahn U et al. Atopic dermatitis is associated with a functional mutation in the promoter of the C-C chemokine RANTES. *Journal of Immunology* 2000; 164: 1612–6.
34. Willemse T. Atopic dermatitis in dogs: current diagnostic criteria. *Tijdschrift voor Diergeneeskunde* 1988; 113: 74–9.
35. Hanifin JM, Lobitz WC. Newer concepts of atopic dermatitis. *Archives of Dermatology* 1977; 113: 663–70.
36. Hanifin JM. Atopic dermatitis. In: Moschella SL, Hurley HJ eds. *Dermatology*, 3rd edn. Philadelphia, Pa: W.B. Saunders Co, 1992: 441–64.
37. Bruijnzeel-Koomen CA, Mudde GC, Bruijnzeel PL. The presence of IgE molecules on epidermal Langerhans cells in atopic dermatitis and their significance for its pathogenesis. *Allergie et Immunologie* 1989; 21: 219–23.

Résumé Bien que la dermatite atopique canine (CAD) soit fréquente, peu de modèles sont disponibles. Le but de cette étude était d'évaluer des beagles haut répondeurs en IgE sensibilisés par voie épicutanée à la poussière de maison (hdm) comme modèle possible de CAD. Six Beagle haut répondeurs en IgE ont été provoqués avec HDM en utilisant des doses variées et des protocoles différents. Des provocations semblables ont été réalisées chez les témoins positifs et négatifs. Trois chiens à DAC et tests cutanés positifs à HDM et trois chiens normaux sans réaction à HDM.

Tous les chiens haut répondeur et les chiens atopiques ont développé des lésions cutanées sévères et un prurit après la provocation. Les lésions consistaient en des papules érythémateuses et des macules sur les zones de contact comme la face, les oreilles, l'abdomen ventral, les espaces axillaires, les ars et les pieds. Les lésions sont apparues dès 6 heures et ont augmenté en sévérité avec le temps. Aucun des chiens sains n'a développé de prurit ou de lésion.

Les biopsies de lésions représentatives ont montré une dermatite périvasculaire avec un infiltrat mononucléé et une spongiose. Des lymphocytes et des éosinophiles s'accumulaient dans des micro abcès épidermiques avec une hyperplasie des cellules dendritiques épidermiques portant des IgE.

Ces données suggèrent que cette colonie de Beagle développe une dermatite qui ressemble cliniquement, microscopiquement et immunologiquement à la DAC spontanée. Il est également conclu que cette modalité de provocation n'est pas irritante pour les chiens sains, mais provoque des poussées chez les chiens atopiques sensibilisés.

Resumen Aunque la dermatitis atópica canina (CAD) es un proceso común, no existen muchos modelos de la enfermedad disponibles. El propósito de este estudio fue evaluar perros de raza Beagle con elevada IgE y sensibilizados por vía epicutánea al ácaro del polvo del hogar, como un posible modelo de CAD. Seis Beagles con elevada IgE fueron expuestos al ácaro del polvo utilizando varias dosis y protocolos. Procedimientos similares se utilizaron en animales utilizados como controles positivos y negativos: tres perros con CAD producida de forma natural y positivos a la prueba intradérmica de la piel para el ácaro del polvo, y tres perros normales sin historia clínica de enfermedad de la piel y negativos mediante la prueba intradérmica al ácaro del polvo.

Todos los Beagles con elevada IgE y todos los perros atópicos desarrollaron lesiones cutáneas severas y prurito tras ser reexpuestos al alérgeno. Las lesiones consistieron en pápulas y máculas eritematosas en áreas de contacto como la cara, orejas, vientre, axilas, ingles y pies. Las lesiones fueron visibles tras 6 horas y aumentaron en intensidad con el tiempo. Los perros normales no desarrollaron lesiones.

Las biopsias de áreas representativas en los Beagles con IgE elevada desarrollaron una dermatitis que según las características clínicas, histopatológicas e inmunológicas semejaron a la enfermedad natural. Por lo tanto concluimos que esta forma de reexposición a alérgeno no es irritante en perros normales pero induce la aparición de lesiones de atopia en perros hipersensibles.

Zusammenfassung Obwohl die canine atopische Dermatitis (CAD) häufig auftritt, gibt es wenige Modelle. Das Ziel dieser Studie war es, Beagles mit hohen IgE Antikörpern, die epikutan zu Hausstaubmilben (HDM) sensibilisiert worden waren, als mögliches Modell für CAD zu evaluieren. Sechs Beagles mit hohen IgE Antikörpern wurden von außen mit HDM in verschiedenen Dosierungen und Protokollen gereizt. Ähnliche Protokolle zur Provokation wurden bei positiven und negativen Kontrollhunden verwendet: drei Hunde mit natürlicher CAD und positivem Intradermaltest (IDT) zu HDM und drei normale Hunde ohne Anamnese einer Hauterkrankung und negativem IDT zu HDM. Alle Beagles mit hohen IgE Antikörpern und alle atopischen Hunde entwickelten starke Hautveränderungen und Juckreiz nach der Provokation. Die Hautläsionen bestanden aus erythematösen Papeln und Maculae in den Kontaktzonen wie Gesicht, Ohren, ventrales Abdomen, Inguinalgegend, Achseln und Füße. Sie waren erstmals nach 6h sichtbar und nahmen an Ausmaß mit der Zeit zu. Keiner der normalen Hunde entwickelte Juckreiz oder Läsionen. Biopsien der jeweiligen Veränderungen wurden von den Beagles mit hohen IgE Antikörpern für histopathologische und immunhistochemische Untersuchungen entnommen. Es bestand eine oberflächliche perivaskuläre Dermatitis mit mononukleären Infiltraten und Spongiose. Lymphozyten und Eosinophile waren in kleinen epidermalen Mikroabszessen angehäuft mit einer Hyperplasie der epidermalen IgE tragenden dendritischen Zellen. Diese Ergebnisse legen nahe, dass diese Kolonie der Beagles mit hohen IgE Antikörpern eine Dermatitis entwickeln, die klinisch, histopathologisch und immunologisch der natürlich vorkommenden caninen Krankheit ähnlich ist. Es wird ebenfalls daraus geschlossen, dass diese Art der Provokation für normale Hunde nicht irritierend wirkt, bei hypersensitiven atopischen Hunden allerdings allergische Schübe auslöst.