

Evaluation of the usefulness of sensitization to aeroallergens as a model for canine atopic dermatitis in genetically predisposed Beagles

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Objective—To evaluate a model for atopic dermatitis (AD) and to measure the effect of sensitization in Beagles genetically predisposed to produce high serum concentrations of allergen specific IgE.

Animals—22 laboratory Beagles.

Procedure—Seventeen dogs were sensitized from birth to 3 allergens (recombinant birch pollen, *Dermatophagoides pteronyssinus*, and *D. farinae*). Five nonsensitized dogs from the same litters served as controls. Clinical scoring, regular intradermal testing, measurement of serum concentrations of allergen-specific IgE, and collection of biopsy specimens of skin at 23, 32, and 43 weeks of age were performed. Serial tissue sections were stained for identification of IgE+ cells, mast cells and their subtypes, T-cells, Langerhans cells, and major histocompatibility complex class-II+ cells. At the age of 15 months, dogs were continuously exposed to 2 µg of mite allergen/g of dust.

Results—Sensitized dogs had positive intradermal test reactions and significantly higher serum concentrations of allergen specific IgE, compared with nonsensitized dogs. In sensitized and nonsensitized dogs, a significantly higher number of mast cells was found at predilection sites, compared with the control biopsy site. The number of mast cells at predilection sites increased with age. Sensitization significantly increased the number of epidermal Langerhans cells by 23 weeks of age. The number of epidermal Langerhans cells significantly increased in nonsensitized dogs by 32 weeks of age. Clinical scoring only revealed mild transient erythema in some dogs.

Conclusions—Increases in concentrations of serum allergen-specific IgE and exposure to allergens is not sufficient to induce clinical signs of AD in genetically predisposed dogs. (*Am J Vet Res* 2002;63:1329-1336)

Atopic dermatitis (AD) is a chronic, highly pruritic, inflammatory skin disease and is the second most common allergic skin disorder in dogs (10% of the canine population).¹ Atopy can be defined as an inher-

ited, increased allergen-specific IgE antibody response to common environmental allergens, such as house dust mites.² Furthermore, immunologic abnormalities, antigenic stimuli, altered physiologic and pharmacologic reactions, and genetic predisposition play a role in the pathogenesis of canine AD.¹

Skin lesions of AD are largely caused by self-trauma brought about by pruritus, secondary bacterial infections, and chronic inflammation. Lesions classically affect the face, feet, distal extremities, flexor surface of the elbow joints and ventrum, or some combination thereof.¹

Histologic features of canine AD consist of a variable degree of a superficial perivascular cell infiltrate composed of mainly lymphocytes and macrophages with a variable number of mast cells (MC) and eosinophils,³ which is similar to that described in humans.⁴ In addition, the composition of the cellular infiltrate is dependent on the chronicity of the skin lesions and whether these are secondary infections.^{4,5}

In humans, the histologic features of AD depend on the phase of the skin lesions.^{4,5} Acute lesions are characterized by a marked perivascular cell infiltrate composed predominantly of T lymphocytes and occasional monocytes or histiocytes. Mast cells in various stages of degranulation are evident in normal numbers.⁶ In more chronic lesions an increased number of Langerhans cells (LC) are evident in the epidermis and an increase in MC is seen in the dermis, but they are generally fully granulated.⁶

High serum IgE concentrations and an increased number of IgE bearing MC, basophils, and LC are important features of AD. Synthesis of IgE by B cells is induced by cytokines of T-helper cells (subset 2), namely interleukin-4 and interleukin-13.⁷

There are several mechanisms by which IgE molecules can contribute to the allergen-induced reactions. It was reported that LC-induced allergen-specific T-cell activation was more efficient when allergen was taken up via IgE bound on LC.⁸ One of the more specific markers for LC are the CD1 (ie, CD1a and CD1c) markers that are part of a third family of antigen-presenting molecules similar to major histocompatibility complex (MHC) class-I and MHC class-II molecules.⁹ After leaving the epidermis, LC undergo developmental and functional maturation and form part of the dermal dendritic cell population. They change from antigen-capturing into antigen presenting cells. Upregulation of MHC class-II molecules, adhesion molecules, and costimulatory markers is associated with these phenotypic changes.¹⁰ The binding of IgE to

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LC has been found in lesional skin and nonlesional skin of canine AD patients but not in healthy control dogs.¹¹

The role of MC in AD is not clear, but there is evidence that they may be important in the development of atopy.^{4,12} Immunoglobulin E and IgG bind to tissue MC, in particular in the skin, the primary target organ of canine atopy. The immunologic activity of MC can be explained by the release of mediators on activation. Mast cells exist as a heterogeneous family of effector cells, and experimental data increasingly indicate that the various functions of MC in skin physiologic and pathologic changes are partially a result of MC heterogeneity. This heterogeneity can be documented as differences in mediator content and in sensitivity to agents that induce activation and mediator release.^{13,14} The main criterion for canine MC subtyping is the content of the MC-specific proteases chymase and tryptase. In dogs, 3 MC subtypes can be distinguished: MC that contain only tryptase, MC that contain tryptase and chymase, and MC that contain only chymase.¹⁵ The functional role of skin tryptase and chymase is not completely known, but it is known that they are involved in epithelial, endothelial, and fibroblast activation and proliferation.¹³ Furthermore, the discovery that MC subtypes produce various cytokines suggests that they interact with other cells of the immune system.

At 1 research facility,¹⁶ more than 200 Beagles were bred and selected for high or low IgE response after sensitization. When these dogs are sensitized from birth with a variety of allergens (recombinant birch pollen [BetV1], milk proteins, peanut, ovalbumin, and several other food allergens) high serum concentrations of allergen-specific IgE may be measured after repeated booster injections of the allergens in the high IgE responder dogs. A certain number of these dogs developed clinical signs comparable to canine AD.¹⁶

These findings encouraged us to breed dogs from the high IgE responder strain and trigger clinical signs of AD by sensitizing them to birch pollen and house dust mites. The aim of our study was to use these dogs as a model for AD in dogs and humans and to investigate disease development.

Materials and Methods

Animals—In our study, 22 high IgE responder Beagles from 4 litters (10 sexually intact females and 12 sexually intact males) were used. The dogs belonged to the research colony of Novartis Centre de Recherche Santé Animale SA, St Aubin, Switzerland. The study was reviewed by an ethics committee according to the Principles of Laboratory Animal Care (National Institute of Health publication 86-23, revised 1985) and the Swiss regulation of animal experiments (Schweizerisches Tierschutzgesetz).

Sensitization procedure—Seventeen dogs (8 sexually intact females and 9 sexually intact males) were sensitized from birth according to a protocol.¹⁶ They received 1 primary subcutaneous injection of BetV1 (1 µg/dog),^a *Dermatophagoides pteronyssinus* (Dp; 500 µg/dog),^b and *Dermatophagoides farinae* (Df; 500 µg/dog),^b diluted in a 1:1 solution of aluminium hydroxide gel^c and 0.9% NaCl within the first 24 hours after birth, followed by 3 subcutaneous

booster injections at a 2-week interval, 2 more booster injections at a 4-week interval, and then 1 booster injection every other month. Five dogs (2 sexually intact females and 3 sexually intact males) were not sensitized and were used as control dogs.

At follow-up evolution at 15 months of age, dogs were continuously exposed to at least 2 µg of mite allergen/g of dust by housing them on carpets infested with Df and Dp mites. One gram of mite culture was added twice weekly to each carpet, and the allergen load was measured with a screening test.^d

Clinical scoring—Dogs were scored weekly for clinical signs of AD by means of a modified canine atopic dermatitis severity index scoring protocol. Scoring was always performed by the same veterinarians (KSE, BS) who were in charge of the clinical part of the experiments. Twenty-four body locations were scored for 4 types of lesions, namely erythema, lichenification, excoriation, and pruritus. Body locations were the following: face, left and right ears, ventral and dorsal aspects of the neck, left and right axillary regions, chest, left and right groin areas, abdomen, left and right forelimbs, left and right hind limbs, left and right front paws, left and right rear paws, lateral aspect of the thorax, lumbar region, dorsal and ventral aspects of the tail, and anus. Points attributed to the 4 types of lesions were 0 for no lesions, 1 for mild lesions, 2 for moderate lesions, and 3 for severe lesions. The highest possible score would, therefore, have been 288 points (24 locations × 4 types of lesions × 3 points). Points from each dog were added to obtain a final result.

Intradermal tests—At the age of 16, 24, 32, and 40 weeks, intradermal tests (IDT) were performed to establish the presence or absence of a type-I hypersensitivity reaction. After clipping the lateral aspect of the abdomen, 0.1 ml of BetV1 in 0.9% NaCl solution (5 µg/ml), 0.1 ml of Dp extract (1:1,000), and 0.1 ml of Df extract (1:1,000) diluted in 0.9% NaCl solution were injected intradermally. These antigen concentrations were chosen on the basis of the results of a dose-titration test in 11 nonsensitized laboratory Beagles. In addition, dogs were given an IV injection of Coomassie blue solution (0.5 ml/kg; 1% wt:vol). Saline (0.9% NaCl) solution diluent served as a negative control and histamine phosphate (0.1 mg/ml) as a positive control. Intradermal tests were evaluated after 20 minutes. The reaction was considered positive when the wheal was blue and the diameter of the wheal was larger than half of the sum of the diameters of the positive and negative control.

Serum allergen-specific IgE and IgG—Blood samples were collected from dogs at weeks 12, 20, 28, 36, 44, and 52, and serum titers of allergen-specific IgE and IgG for BetV1, Dp, and Df were measured semi-quantitatively by an ELISA.^e

Biopsy specimens—To evaluate histopathologic changes, 8-mm-diameter punch biopsy specimens of skin were taken from dogs under general anesthesia (medetomidine hydrochloride, 0.04 ml/kg, IV; ketamine hydrochloride, 0.05 ml/kg, IV). Biopsy specimens were obtained at 3 time points (23, 32, and 43 weeks of age) from 4 predilection sites for AD (lower lip, flexor surface of the elbow joint, dorsal interdigital space of the front paw, and inner aspect of the thigh) and 1 control site (back). Each biopsy specimen was cut in half. One half was fixed in buffered 4% formalin at room temperature (RT; approx 20 °C) for 24 hours and embedded in paraffin wax. The second half of the biopsy specimen was embedded in a compound to bind tissue to the specimen block^f and frozen in liquid nitrogen.

Formalin-fixed tissue—Serial tissue sections were cut at a thickness of 4 µm. One section was stained with H&E and

1 with toluidine blue for detection of the sulfated acid glycosaminoglycans in the MC granules. The neutral protease content of MC was examined with an enzyme-immunohistochemical staining technique. An enzyme-histochemical reaction was used for the detection of chymase activity, and an immunohistochemical staining method with a polyclonal antibody was used for the detection of tryptase (double-labeled MC). The specificity of these staining techniques under standardized fixation and staining conditions for the detection of canine and bovine MC was evaluated and reported previously.^{15,17} The enzyme-histochemical reaction for the detection of chymase was performed with a commercially available detection kit⁸ with naphthol-AS-D-chloracetate as substrate. Immunohistochemistry for tryptase was performed with a polyclonal rabbit anti-human skin tryptase antibody at a dilution of 1:1,500.¹⁸

Immunoglobulin E+ cells were identified with canine IgE-specific antibodies raised in chickens¹⁹ by means of the alkaline phosphatase-antialkaline phosphatase method.^h Deparaffinized and rehydrated tissue sections were digested with 0.1% pronase E for 5 minutes at 37 C. After incubation with the canine IgE-specific antibody (1:100; 30 minutes; RT) a mouse anti-chicken IgGⁱ (1:100; 30 minutes; RT) was applied. This step was followed by incubation with a bridging antibody (1:100; 30 minutes; RT) and consecutively with the alkaline phosphatase-antialkaline phosphatase complex (1:100; 30 minutes; RT). Incubation with the mouse anti-chicken IgG and the alkaline phosphatase-antialkaline phosphatase complex for each sample was performed twice.

Frozen tissue—Serial sections (4 µm thick) of the frozen tissue were used for the detection and quantitation of T cells (CD3+), LC (CD1c+), and other antigen-presenting cells (MHC II+). Murine monoclonal antibodies^j (1:50; 12 h; 4 C) were used to detect CD1c and MHC II. A rabbit anti-human T-cell antibody^k (1:1,000; 12 h; 4 C) was used to identify CD3. Immunohistochemistry for the labeling of these 3 antigens was performed as follows: after fixation in acetone for 7 minutes, the sections were incubated in 0.3% hydrogen peroxide in 0.1% [wt:vol] sodium azide for 10 minutes at RT to quench endogenous peroxidase activity. Nonspecific binding sites were blocked with horse serum (20% [wt:vol]; 20 minutes; RT). For the detection of the primary antibody of MHC II and CD1c, a biotinylated goat anti-mouse IgM^l (1:200; 30 minutes; RT) was applied followed by incubation with avidin and biotinylated peroxidase complex^m (1:200; 20 minutes; RT). After the application of the substrate-chromogenⁿ mixture the sections were counterstained in hemalum. Between each step the sections were washed 3 times with Tris-buffered saline solution for 3 minutes and CD3+ cells were identified with a commercially available kitⁿ according to the manufacturer's protocol.

Skin specimens were investigated by histologic evaluation of the H&E slides, determination of mean MC density/mm² by use of the toluidine-blue staining method, determination of mean MC density/mm² by use of the enzyme-immunohistochemical double-labeling technique for MC proteases, and

determination of density and percentage distribution of the MC subtypes according to their protease content. In the epidermis and dermis, mean density of IgE+ cells/mm², mean density of CD1c+ cells/mm², mean density of MHC II+ cells/mm², and mean density of CD3+ cells/mm² were determined as well as the epidermis-to-dermis ratio of cell densities for each cell type.

Histologic evaluation of the H&E stained slides was performed by use of a semi quantitative grading system (0, 1, 2, 3) to assess the degree (none, mild, moderate, or severe) of cellular infiltration (ie, lymphocytes, mononuclear cells, eosinophils, MC) in the dermis. Changes in the epidermis (increased epidermal thickness, spongiosis, and cellular infiltration) were similarly graded. The final score for each biopsy specimen was the mean of the assessed grade for the dermis and the grade for the epidermis.

Quantitation of positively stained cells was performed by evaluation of the total area of each section by use of a square eyepiece graticule (objective × 25 magnification, eyepiece × 10 magnification, squares [10 × 10] with a total area of 0.0156 mm²). Only positively stained cells with a visible nucleus were counted. Positively stained cells in the wall of hair follicles were not included.

Cell densities and the percentage distribution of cell types were compared among body locations at each time point. Furthermore, changes of each cell type were monitored over time. Results of sensitized and nonsensitized dogs were compared.

Statistical analysis—Analysis of variance was performed by use of a computer program.^o A value of $P < 0.05$ was considered significant. Immunoglobulin E and IgG concentrations were compared by Mann-Whitney U tests. Statistics for CD3+, CD1c+, and MHCII+ cells was performed by use of Kruskal-Wallis and Mann-Whitney U tests. The number of IgE+ cells, toluidine-blue stained MC, and double-labeled MC were analyzed by the Tukey test.

Results

Clinical scoring—Weekly scoring of dogs for clinical signs of AD revealed transient mild erythema, mainly observed at locations such as the face, ears, ventral aspect of the neck, chest, groin area, and interdigital skin. These lesions were detected in sensitized and control dogs to almost the same extent. The highest value was 20 for sensitized dogs (mean weekly values ranged from 2 to 14.2) and 16 for nonsensitized dogs (mean weekly values ranged from 0.4 and 9). Lesions appeared and disappeared on an irregular basis and became neither pruritic nor chronic. Clinical investigators never observed dogs scratching or licking, nor did they observe self-induced alopecia.

Intradermal tests and serumconcen of allergen-specific IgE and IgG—Most sensitized dogs had a positive skin test reaction for BetV1, Dp, and Df after 20 minutes.

Table 1—Number of positively reacting sensitized (n = 17) and nonsensitized dogs (5) 20 minutes after intradermal testing with 3 antigens at various ages

Age (w)	Recombinant birch pollen		Dermatophagoides pteronyssinus		Dermatophagoides farinae	
	Sensitized dogs	Nonsensitized dogs	Sensitized dogs	Nonsensitized dogs	Sensitized dogs	Nonsensitized dogs
16	16	0	16	0	14	0
24	14	0	14	1	14	1
32	17	0	17	4	16	4
40	17	3	17	5	17	5

At 16 weeks of age, all nonsensitized dogs had a negative skin test reaction for injected antigens. However, positive skin test reactions were observed with increasing age of dogs (Table 1). Serum titers of allergen specific IgE and IgG were significantly higher ($P < 0.001$) in sensitized dogs, compared with nonsensitized dogs (Fig 1).

Dermatopathologic findings—Most dogs had mild, regular hyperplasia of the epidermis and spon-

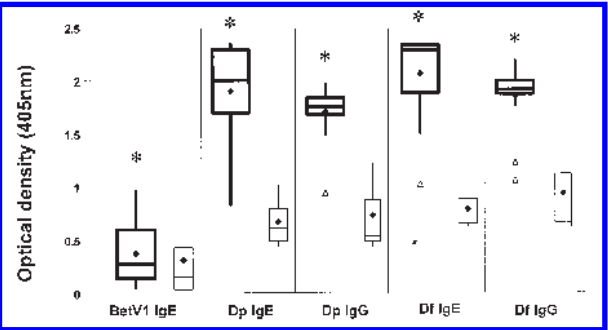


Figure 1—Boxplots representing the mean values for serum antigen-specific IgE and IgG in sensitized (bold lines) and nonsensitized (thin lines) dogs. Notice mean (filled diamond) and high or low (open triangle; greater than 1.5 times the height of box) values. *Significantly ($P < 0.05$) different from nonsensitized dogs. BetV1 = Recombinant birch pollen. Dp = *Dermatophagoides pteronyssinus*. Df = *Dermatophagoides farinae*.

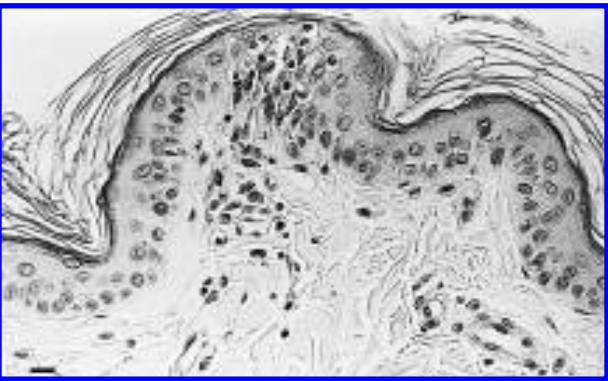


Figure 2—Photomicrograph of a section of a skin biopsy specimen from the flexor surface of the elbow joint of a sensitized dog. Notice the focal spongiosis and the mild perivascular infiltration composed of mast cells, Langerhans cells, and mononuclear cells in the dermis. H&E; bar = 24 μ m

giosis (Fig 2). In the dermis, a mild perivascular infiltration composed of MC, lymphocytes, and mononuclear cells was present. These mild pathologic changes were seen in biopsy specimens of atopy predilection sites and the control site in biopsy specimens taken from sensitized dogs. The mean score obtained from sensitized dogs was 1.1. The mean score obtained from nonsensitized dogs was 0.4. One sensitized and 1 nonsensitized dog had a biopsy specimen with mural folliculitis and *Demodex* mites in the lumen of a hair follicle at week 23.

Enzyme and immunohistochemical analysis—Sensitized dogs had a significantly higher density of IgE+ cells ($P = 0.002$) in the dermis, compared with nonsensitized dogs. No IgE+ cells were found in the epidermis. Sensitization did not influence the number of MC or MC subtypes. Mean values for the number of MC stained with toluidine blue, total number of MC subtypes after double labeling, percentage distribution of the MC subtypes, and the number of IgE+ cells at various body locations were determined (Table 2). In 3 (lower lip, flexor surface of the elbow joint, paw) of 4 predilection sites for AD (lower lip, flexor surface of the elbow joint, paw, and inner thigh) the density of all cell types investigated was significantly ($P < 0.001$) higher, compared with the control site. Cell density in the inner thigh was comparable to the back (toluidine-blue MC, $P = 0.188$; double-labeled MC, $P = 0.735$; IgE+ cells, $P = 0.793$). The percentage of the MC subtypes did not differ among various locations.

Comparison of the cell densities in biopsy specimens at various time points revealed an increase of MC stained with toluidine blue (Fig 3), double-labeled MC (Fig 4), and IgE+ cells (Fig 5) between weeks 23 and 32. Furthermore, the density of each MC subtype increased during this time. A significant increase of cell density was also observed between weeks 23 and 43 for MC but not for IgE+ cells. Comparison of weeks 32 and 43 only revealed a significant increase of toluidine-blue stained MC and MC that contained only tryptase (Table 3).

Sensitization had a significant effect on the number of CD1c+ cells in the epidermis in all biopsy sites at week 23. In the dermis the number of CD1c+ cells was not increased. Furthermore, the epidermis-to-der-

Table 2—Mean values of cell density and percentage distribution of mast cell subtypes in biopsy specimens of skin obtained from 5 body locations in Beagles

Cell type	Control site	Predilection site				
	Back	Paw	Lip	Flexor surface elbow joint	Inner aspect thigh	
Toluidine-blue stained MC (cells/mm ²)	30.80	51.10	43.00	43.30	34.00	
Double-labeled MC (cells/mm ²)	25.60	43.40	35.10	34.50	27.30	
IgE+ (cells/mm ²)	19.3	31.40	27.40	27.60	20.60	
T-MC (cells/mm ²)	1.42	2.41	2.10	2.09	1.50	
TC-MC (cells/mm ²)	22.50	37.70	30.80	30.30	23.50	
C-MC (cells/mm ²)	1.16	2.43	1.78	1.95	1.52	
T-MC (%)	5.60	5.60	6.00	6.10	5.60	
TC-MC (%)	88.90	88.10	88.20	88.00	88.00	
C-MC (%)	4.60	5.70	5.10	5.60	5.70	

MC = Mast cells. T-MC = Mast cells that contain only tryptase. TC-MC = Mast cells that contain tryptase and chymase. C-MC = Mast cells that contain only chymase.

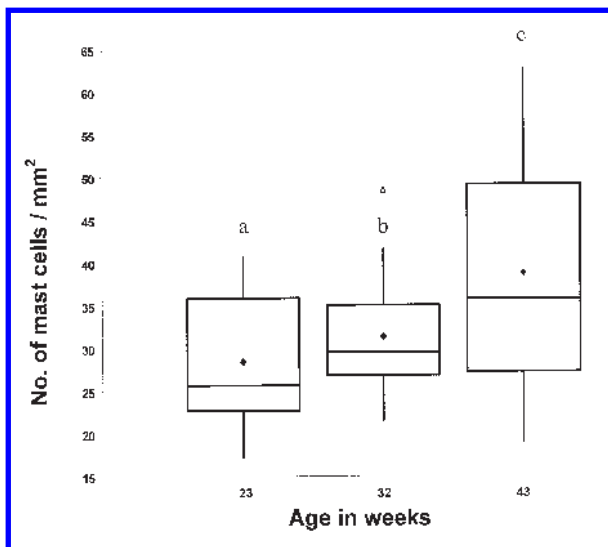


Figure 3—Boxplots representing the density of mast cells stained with toluidine blue in skin biopsy specimens from Beagles at various ages. ^{a,b,c}Differing superscript letters indicate significant ($P < 0.05$) differences among ages. See Figure 1 for remainder of key.

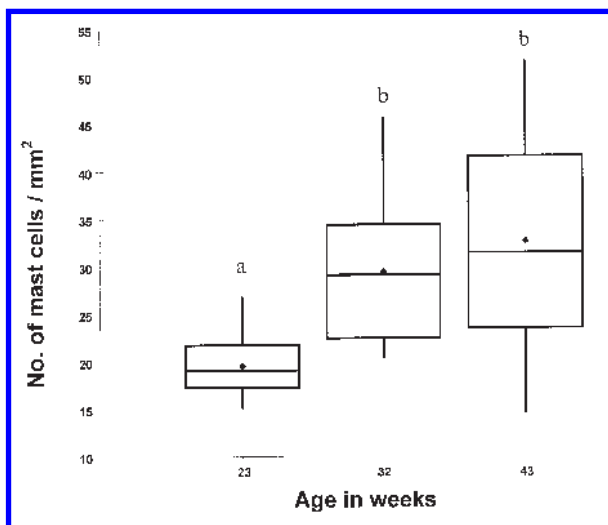


Figure 4—Boxplots representing the density of double-labeled mast cells in skin biopsy specimens from Beagles at various ages. ^{a,b}Differing superscript letters indicate significant ($P < 0.05$) differences among ages. See Figure 1 for remainder of key.

mis ratio of CD1c+ cells and CD3+ cells was significantly increased in all biopsy sites in the sensitized dogs at week 23 (Table 4). The MHCII+-to-CD3+ cell ratio in the dermis was significantly increased as well at both time points in sensitized dogs, compared with nonsensitized dogs (Fig 6). The density of CD3+ cells, however, was significantly higher in nonsensitized dogs at weeks 23 and 32 (Fig 7), compared with sensitized dogs.

Comparison of the predilection sites versus the back revealed a significant increase in the number of CD1c+ cells and MHCII+ cells in the dermis in some of the predilections sites, primarily in the nonsensitized dogs at week 32. Comparison of mean values for all biopsy sites at various time points revealed a signifi-

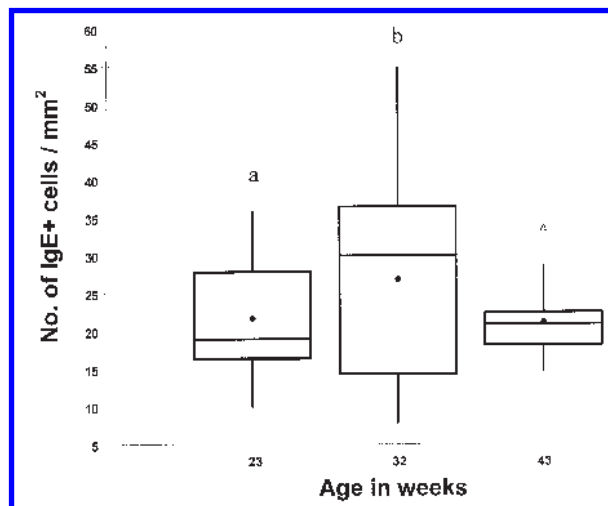


Figure 5—Boxplots representing the density of IgE+ cells in skin biopsy specimens from Beagles at various ages. ^{a,b}Differing superscript letters indicate significant ($P < 0.05$) differences among ages. Differences were not evaluated between weeks 23 and 43 or between weeks 32 and 43. See Figure 1 for remainder of key.

Table 3—Comparisons on the basis of age of cell density and percentage distribution of mast cell subtypes in biopsy specimens of skin obtained from Beagles

Cell type	P values		
	23 vs 32 wk	23 vs 43 wk	32 vs 43 wk
Toluidine-blue stained MC (cells/mm ²)	0.002	< 0.001	< 0.001
Double-labeled MC (cells/mm ²)	< 0.001	< 0.001	0.536
IgE+ (cells/mm ²)	0.021	0.509	0.260
T-MC (cells/mm ²)	< 0.001	< 0.001	< 0.001
TC-MC (cells/mm ²)	< 0.001	< 0.001	0.980
C-MC (cells/mm ²)	< 0.001	< 0.001	0.760
T-MC (%)	0.015	< 0.001	< 0.001
TC-MC (%)	< 0.001	< 0.001	< 0.001
C-MC (%)	< 0.001	< 0.001	0.979

$P < 0.05$ considered significant.
See Table 2 for key.

Table 4—Comparisons on the basis of location and age of the density of various cell types in biopsy specimens of skin obtained from Beagles

Location	Week	P values		
		CD1c+	CD3+	MHC II+
Epidermis	23	< 0.001*	1.000	0.506
	32	0.279	0.048†	0.574
Dermis	23	0.442	0.004†	0.279
	32	0.959	0.004†	0.064
Epidermis and dermis	23	< 0.001*	0.019*	0.721
	32	0.082	0.364	0.574

*Sensitized dogs had a higher cell density, compared with nonsensitized dogs. †Nonsensitized dogs had a higher cell density, compared with sensitized dogs. MHC II = Major histocompatibility complex class II.
 $P < 0.05$ considered significant.

cant increase in the number of CD1c+ cells in nonsensitized dogs at week 32. Comparison of mean epidermis-to-dermis ratios for all biopsy sites at the various time points revealed a higher epidermis-to-dermis ratio of CD1c+ cells at week 32 for sensitized and nonsensitized dogs.

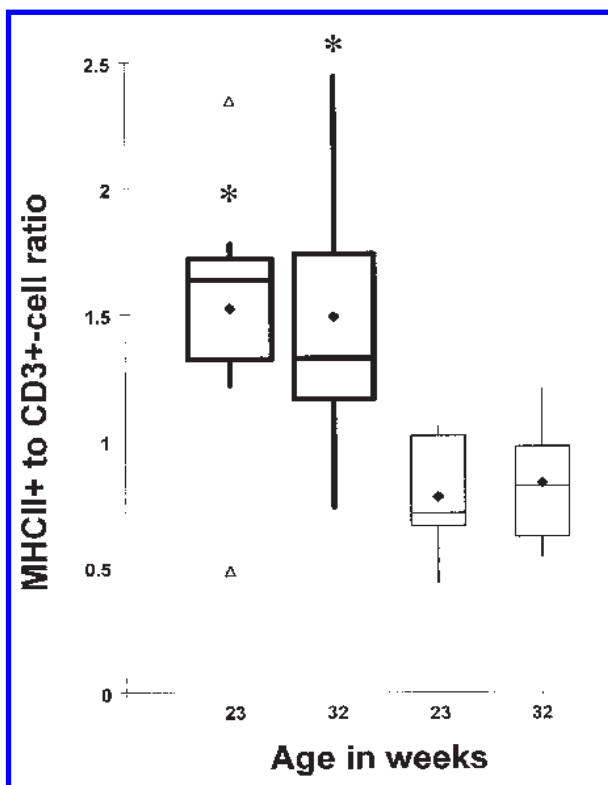


Figure 6—Boxplots representing the MHCII+ to CD3+ cell ratio in the dermis of sensitized and nonsensitized dogs at 23 and 32 weeks of age. *At each age, significantly ($P < 0.05$) different from nonsensitized dogs. See Figure 1 for remainder of key.

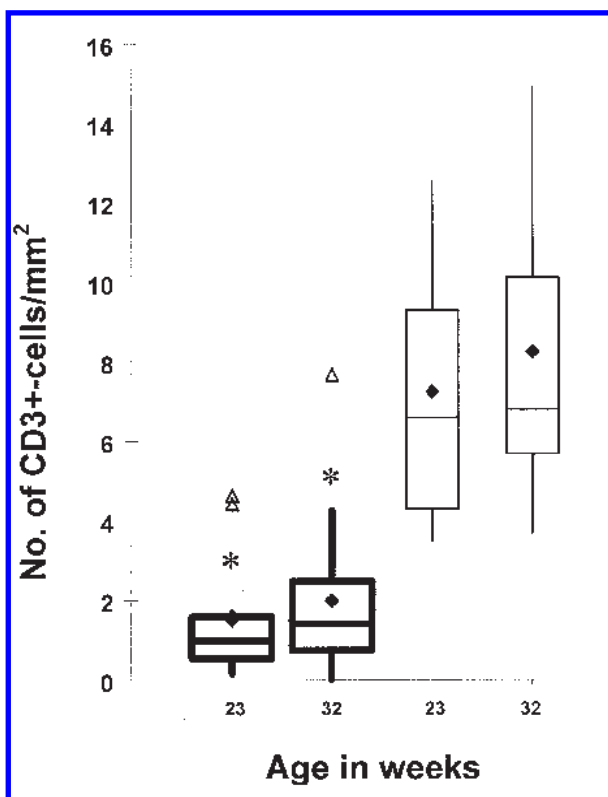


Figure 7—Boxplots representing the density of CD3+ cells in the dermis of sensitized and nonsensitized dogs at 23 and 32 weeks of age. See Figures 1 and 6 for remainder of key.

Discussion

Our study was performed to induce clinical and histologic signs of AD in high-IgE responder dogs and to monitor disease development. In our study, high serum titers of Dp-, Df-, and BetV1-specific serum IgE and IgG antibodies were detectable in all sensitized dogs. Nonsensitized dogs had significantly lower serum titers. This is in agreement with the previously observed findings in the high-IgE-response founder dogs of our litters.¹⁶ In 80% of dogs and humans with AD, serum IgE concentrations are high with a specificity to a variety of aeroallergens, and affected individuals demonstrate an immediate-type skin reaction.²⁰

More than 80% of our sensitized dogs had an immediate-type hypersensitivity reaction, shown by IDT independent of the age of the dog. In nonsensitized dogs, the IDT results varied depending on the age of the dogs. In the 16-week-old dogs, none of the dogs had an immediate-type hypersensitivity reaction. However, with increasing age the percentage of positive reacting dogs increased, and at 40 weeks of age all dogs had an immediate-type hypersensitivity reaction. This finding may have been caused by the repeatedly performed IDT and the fact that the nonsensitized control dogs were high-IgE responder dogs. This combination may have induced positive skin test reactions. Although positive IDT reactions can also be caused by irritant reactions initiated by improper preparation and administration of allergenic solutions, it is unlikely that irritant reactions were the cause. The IDT were performed in all dogs in the same way and with the same preparations, and positive reactions were not observed at the beginning of the trial. Furthermore, we tested various dilutions of Dp and Df (1:10²; 1:10³; 1:10⁴; 1:10⁵ and 1:10⁶) prior to this experiment in nonsensitized laboratory Beagles and did not observe any positive reactions at a dilution of 1:1,000 after 20 minutes.

Positive skin test reactions could also be explained by cross-reactivity with other mite allergens, such as storage mites or other house dust mites from food sources. Cross reactivity between mite allergens has long been recognized in small animals and humans, and the time of exposure to such cross-reacting allergens might be important. Positive intradermal test reactions to Df were observed in 12 of 20 dogs with sarcoptic mange.²¹ Moreover, in humans, strong cross reactivity was found between *Otodectes cynotis* and *Dermatophagoides* spp in 7 patients who were allergic to house dust mites.²² In a recent study however, detection of house dust mites could not be confirmed in dried dog food stored under various conditions.²³

Although we were able to induce increased allergen-specific IgE titers and positive IDT reactions, none of the sensitized dogs developed pruritus, which is considered the hallmark of AD. The only clinical sign we observed was mild and transient erythema at the atopy predilection sites. These signs were detected to almost the same extent in sensitized and nonsensitized dogs.

Histologic examination of the skin biopsy specimens from sensitized dogs revealed mild, superficial perivascular dermatitis composed of MC, lymphocytes,

and mononuclear cells. In some instances the epidermis was spongiotic. These findings are mild yet similar, although not specific, to those reported for humans⁶ and dogs with AD.^{3,11,24} The number of IgE+ cells in the dermis was significantly higher in sensitized dogs, compared with the nonsensitized dogs. The difference between the number of IgE+ cells and the number of MC may indicate that dendritic cells are involved in allergen uptake. It has already been reported by other authors that epidermal (ie, LC) and dermal dendritic cells in the skin of dogs with AD express surface IgE, whereas IgE expression could not be detected in the skin of control dogs.²⁵ Interestingly, we could not detect IgE+ cells in the epidermis of sensitized dogs. Epidermal LC with surface bound IgE are believed to play a role in facilitated allergen capture and presentation in human AD²⁶ and canine AD and primary sensitization to allergens occurs through the skin.²⁷ This could be a reason why housing dogs on carpets infested with Df and Dp did not induce clinical signs of AD.

No significant difference was detectable concerning the MC density in the dermis between the sensitized and the nonsensitized dogs, independent of the staining technique. In agreement with these findings is a study of AD in humans, where MC numbers are normal if detected by metachromatic staining procedures.²⁸ In a study of MC density in the skin of dogs with AD, approximately the same MC density after toluidine-blue staining was detected in the skin of atopic and control dogs.²⁹ However, the authors report that, after double labeling a significantly lower MC density was observed in lesional and nonlesional skin of atopic dogs. These findings are therefore most likely the result of differing disease stages.

In our study, the MC density increased significantly with age, independent of the staining technique applied. Furthermore, the percentage distribution of each MC subtype did not change significantly with age. The apparent increase of MC with age might be a result of MC upregulation as a result of changes in the microenvironment of the skin. Because sensitization did not have any effect on the number of MC, this might be a consequence of the age of the dogs. On the other hand, it is possible that most IgE+ cells are MC that are degranulated and could not be detected by our staining techniques.

Investigation of the MC density in the skin of various body locations revealed a significantly higher number of MC in certain predilection sites for AD. Similar results were obtained by Auxilia and Hill.³⁰ The authors found significantly higher MC densities at the pinnae and ventral interdigital skin of the feet, compared with the other body locations they examined. They suggested that cutaneous MC distribution might be a reason for the frequent occurrence of clinical signs in certain locations.

In contrast to what has been described in lesional and nonlesional skin of dogs with AD we could not detect a significant increase in the number of LC, T lymphocytes, or MHC class-II+ cells in sensitized dogs at week 32.^{11,25,31} This finding is in accordance with the lack of clinical signs of AD in sensitized dogs in our study.

Willemse³² suggested guidelines for the diagnosis of AD.³² According to that report, at least 3 of the following major features should be evident in AD: pruritus, facial or digital involvement, involvement of the flexor and extensor surface of joints, chronic or chronically relapsing dermatitis, an individual or family history of atopy, and a breed predilection. Furthermore, 3 of the following minor features should also be evident: 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 concentrations, and high allergen-specific IgE concentrations.

Dogs in our study manifested 3 of the minor features, namely immediate skin test reactivity, high allergen-specific IgE concentrations, and high allergen-specific IgG concentrations, but only 1 of the major features (family history of atopy) of AD. Therefore, the study reported herein indicates clearly that the mere detection of allergen-specific IgE, and exposure to the allergen, is not sufficient to produce clinical signs of AD in genetically predisposed dogs.

It is known that in humans and dogs with AD secondary bacterial infections or infections with *Malassezia* spp can exacerbate AD.^{33,34} Therefore, we investigated dogs for infestation with *M pachydermatis* by tape strips and fungal cultures but could not detect any yeasts. The lack of *M pachydermatis* or superficial pyoderma may be the missing link in development of a dermatitis reaction as it has been reported for other high IgE-responder dogs.³⁵

Furthermore, our findings must cast some doubts on the value of IDT and measurement of allergen-specific IgE to house dust mites in the diagnosis of AD. Intradermal testing has long been considered the standard for allergy testing in dogs. Undoubtedly the IDT is a sensitive method. However, it has been reported earlier that cats and dogs may be positive to 1 or more allergens even without evidence of allergic disease.³⁶ Halliwell et al³⁷ showed that Df-specific IgE was commonly found in clinically normal dogs. This finding was generally associated with a positive IDT reaction. However, the same author reported that laboratory-reared dogs had negative IDT reactions and no allergen-specific IgE.³⁷ In their attempt to explain why some dogs with IgE to house dust mite allergens develop disease whereas others do not, Halliwell et al³⁷ suggest that perhaps it is the amount of IgE that is relevant. Alternatively, IgE may be functionally heterogeneous, the MC receptor may be heterogeneous; or IgE may not be involved in the pathogenesis of AD. A study on histamine release from basophils of atopic and clinically normal dogs supports a possible heterogeneity of either IgE or of the MC receptor.³⁸ In this report the total cell histamine did not differ significantly in a group of 12 atopic dogs, compared with 5 laboratory-reared Beagles. Total serum IgE was also not significantly different in the 2 groups. Yet histamine release in response to anti-IgE was significantly greater in the atopic dogs. Furthermore, after immunization with Df allergen, the concentration of allergen-specific IgE was greater in the clinically normal dogs than in the atopic dogs. However, histamine release in response to the aller-

gen was only found in the atopic dogs. These findings could be explained by heterogeneity of either IgE or the MC receptor. Although functional heterogeneity of canine IgE has yet to be documented, physiochemical heterogeneity has been reported.³⁹

^aBiomay, Linz, Austria.

^bGreer Laboratories, Lenoir, NC.

^cServa, Heidelberg, Germany.

^dCMG-HESKA Allergy Products, Fribourg, Switzerland.

^eImovet CAC Labor Laupeneck, Bern, Switzerland.

^fTissue Tek, Sakura Finetek Europe BV, Zoeterwoude, The Netherlands.

^gSigma Chemical Co, St Louis, Mo.

^hDianova, Hamburg, Germany.

ⁱSigma, St Louis, Mo.

^jclone: CA2.1C12 and clone CA13.9H11, provided by Peter F Moore, University of California, Davis, Calif.

^kCode No. A 0452, Dako Diagnostics AG, Zug, Switzerland.

^lJackson, West Grove, Pa.

^mVectastain Elite ABC Kit, Vector Laboratories Inc, Burlingame, Calif.

ⁿAEC, Dako Diagnostics AG, Zug, Switzerland.

^oLSAB 2 System, Dako Diagnostics AG, Zug, Switzerland.

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