

Transmission electron microscopy studies in an experimental model of canine atopic dermatitis

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Conflict of Interest

No conflicts of interest have been declared.

Abstract

Impairment of skin barrier function has been hypothesized in canine atopic dermatitis (AD). In this prospective, controlled study, the ultrastructure of the upper epidermal layers was investigated using an experimental model of canine AD. Seven atopic Beagles sensitized to *Demodex phagocytes* and four healthy Beagles were used as controls. Both normal and atopic dogs were challenged with *D. phagocytes* for 3 days. Clinical signs were scored and skin biopsies were taken from the inguinal area before and 3 days after allergen exposure. Samples were processed to enhance lipid visibility and evaluated by Transmission Electron Microscopy. Emphasis was placed on evaluation of the lipid lamellae (LL), and lamellar bodies (LB) of the stratum corneum.

After allergen challenge, atopic Beagles developed severe pruritic dermatitis while no skin lesions were noted in the controls. Ultrastructurally, before allergen challenge, atopic Beagles displayed focally severe abnormalities in LL organization and wider intercellular spaces containing abnormal lipid material. In atopic Beagles, LBs were frequently found inside corneocytes while this finding was not observed in the controls. After allergen challenge, further increase of intercellular spaces was observed in the stratum corneum of atopic Beagles while no appreciable changes were observed in the normal dogs. Intercellular spaces in atopic Beagles were filled with abundant amounts of abnormal lipid material and highly disorganized LL. It is concluded that baseline differences in the ultrastructure of the skin exist between normal and experimentally sensitized atopic Beagles and that these changes are aggravated by allergen challenge and the resulting flare-up of dermatitis.

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Introduction

Canine atopic dermatitis (AD) is the second most common allergic skin disease, affecting approximately 10% of the canine population.¹ It has been defined as a genetically inherited inflammatory skin disease with characteristic clinical features, most commonly associated with hypersensitivity to environmental allergens.² The understanding of its pathogenesis is limited: most of the research on canine AD so far has focused on the aberrant immunological responses rather than the barrier function of the skin in this disease.

Skin barrier function is largely effected by the lower part of the stratum corneum and is dependent on its structural integrity.³ Such integrity is maintained by modified desmosomes, terminally differentiated keratinocytes, and intercellular lipids.⁴ During the process of keratinization, organelles contained in the stratum granulosum called lamellar bodies (LB) are instrumental in providing the necessary lipids and enzymes for further differentiation and ultimate desquamation of the epidermis.⁴ The lipids provided by the LB are then organized to form the extracellular lipid lamellae (LL), which help prevent the loss of internal water and limit the penetration of exogenous material (e.g. allergens). The LL matrix is typically composed of a variety of lipids including ceramides, cholesterol, fatty acids and cholesterol esters.⁵

Impairment of the skin barrier has been extensively studied in humans with AD.^{6,7}

It is known that, in humans with AD, a genetically impaired skin barrier function is present in both lesional and nonlesional skin. Humans with AD also have marked alterations in the amount and composition of ceramides⁸ and impairment in the extrusion of LB.^{9,10} More specifically, the release of LB is incomplete and retarded and LB can be easily found in the stratum corneum. A disturbed differentiation, including changes in lipid and protein composition and content and an impaired extrusion of LB, has been demonstrated to cause an impaired barrier function in humans. It is hypothesized that this defective permeability allows an enhanced penetration of environmental allergens, thus increasing the risk for sensitization in genetically predisposed individuals.^{11,12} Allergic sensitization triggers an inflammatory process, which further impairs skin barrier function. This leads to self-perpetuating cycles of additional sensitizations and progressive worsening of the disease.

Canine AD shares many similarities with the human counterpart, both clinically and immunologically.¹³ Limited studies have been done so far to investigate the role of barrier function in canine AD. It is known that the epicutaneous route of allergen exposure plays an important role in the canine disease¹⁴ and abnormalities of both shape and structure of stratum corneum LL were reported in atopic dogs suggesting a defect in epidermal lipids.¹⁵ Whether these changes are primary or secondary and how allergen exposure may modulate LL is unknown. Some of these questions are difficult to address using dogs with naturally occurring disease due to the fact that great variation may exist in the barrier function of different breeds, and the age of lesions observed clinically is unknown. Barrier function is greatly affected by environmental conditions (e.g. temperature, humidity), dietary intake, stage of the disease, and topical therapy (type and frequency). It would be challenging to control for all these factors using animals with naturally occurring AD. An experimental model, in which lesions can be directly induced and monitored and where environmental and dietary conditions are strictly controlled, would be of great benefit.

In the past, unsuccessful attempts had been made to reproduce clinical disease by simply selecting for high IgE responders.¹⁶ An experimental model for canine AD has been identified in a colony of Beagles epicutaneously sensitized to *Dermatophagoides farinae*.¹⁷ These dogs consistently develop skin lesions and pruritus upon environmental exposure to the allergen of reference [house dust mite, HDM (pure culture of *Dermatophagoides farinae*)].¹⁷ The advantages of having access to this model for this initial investigation are multiple. Besides being able to control external factors that may affect barrier function, derangements of the barrier function could be evaluated in a sequential manner to determine whether alterations in the barrier are primary or secondary to inflammatory changes induced by allergen challenge or both. The clinical implications of ultrastructure changes could then be evaluated by correlating them with clinical signs.

The purpose of the present study was to evaluate by electron microscopy the ultrastructure of the upper layers of the epidermis in this experimental model of canine AD, to compare it to normal Beagles, and to investigate the effect of HDM exposure. It was hypothesized that ultrastructural derangements occur as a result of allergen exposure.

Experimental design and methods

All Animal procedures of this study were approved by the Institutional Animal Care and Use Committee of the University of Florida.

Animals and housing conditions

Seven atopic Beagles and four healthy Beagles (normal controls) were used for this study. The atopic Beagles had been previously epicutaneously sensitized to HDM and were known to have positive atopy patch test and positive intradermal skin test reactions at 15 min to HDM (25 PNU/mL, mixture of *Dermatophagoides farinae* and *D. pteronyssinus*, Greer Laboratories Inc., Lenoir, NC, USA). Reactions to intradermal skin test with HDM were subjectively scored as 4+ in all the dogs in previous studies (0 being the saline score and 4+ being the histamine score, negative and positive control respectively). These dogs were also known to develop skin lesions

and pruritus upon allergen exposure to HDM.¹⁷ Biopsies of these lesions had demonstrated that they are histologically and immunologically compatible with naturally occurring canine AD.¹⁷

The healthy controls were selected to match the age of the high IgE Beagles. These dogs were selected based on the lack of history of skin disease, lack of evidence of skin lesions on physical examination, lack of reactivity after intradermal injection of HDM and negative serology test results. The controls were obtained from another research colony housed at the same institution.

All dogs were housed in the same research facility at the University of Florida. Dogs were housed in individual cement runs that 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 these runs. Walls and air filters were checked monthly to ensure the absence of dust mites in the environment in between experiments. Results were always below the detection level of the test used (MITE-T-Fast™ Allergen Detection System; AVEHO Biosciences Inc., Monterey, TN, USA). Prior to the beginning of the experiments here described, the normal Beagles had not been challenged with the allergen for over a year prior and the atopic Beagles had not been exposed to the allergen for 4 months.

Allergen challenge

All atopic Beagles were environmentally challenged with HDM, as previously described.¹⁷ Briefly, HDM was prepared from a HDM culture (natural *D. farinae*, Greer Laboratories Inc.), and mixed with filtered (0.2 µm syringe filter) phosphate buffered saline (PBS) (pH 7.2) to obtain a final concentration of 31 mg/mL. The solution was vortexed and sonicated prior to use. The HDM solution (1.6 mL/dog/challenge) was applied daily for 3 days on the floor of a plastic air kennel. Each dog was placed in the kennel for 3 h/day, for three consecutive days. The concentration and volume of HDM used were selected based on previous studies.¹⁷

To control for potential effects of HDM on the skin barrier even in the absence of an allergic reaction, healthy Beagles were similarly challenged and biopsies were obtained. Although an effect on barrier function by *D. farinae* has not been previously reported, it is known that *D. pteronyssinus* can disrupt tight junction in epithelia and facilitate allergen delivery,¹⁸ thus allergen challenge of the normal dogs was deemed necessary.

Clinical evaluation

All dogs were evaluated and scored before and on day 3 of allergen exposure. Clinical signs were evaluated by the investigator using a validated scoring system (Canine Atopic Dermatitis and Extent Severity Index 03, CADESI).¹⁹ Briefly, the dog's body was divided into small sections, each of which received a score based on the clinical signs evaluated. The total score was calculated by adding the scores of all clinical signs and body sites. Total score was used for analysis. Clinical signs evaluated included diffuse erythema, erythematous macules, papules, excoriations, and alopecia. The scoring system ranged from zero (absent) to three (severe) for each site and each sign.

Skin biopsies

Two 6 mm-biopsies were taken from each dog before allergen challenge (baseline biopsy from nonlesional area) and on the third day of challenge, 3 h after the last allergen exposure. The second biopsy was taken from lesional skin in the atopic Beagles. All samples were taken from the inguinal area to minimize variations due to body location. The inguinal area was selected due to the fact that it is easy to biopsy and consistently shows lesions after allergen challenge in atopic Beagles.¹⁷ The third day time frame was selected based on previous studies showing that both skin lesions and pruritus reach their peak on the third day of allergen challenge.^{14,17} Biopsies were taken after local anaesthesia with lidocaine, sutured routinely and processed for Transmission Electron Microscopy (TEM) at the University of Florida EM Core Laboratory.

Transmission Electron Microscopy

Skin samples were placed in PBS and refrigerated overnight. The time before processing was consistent in each group. To enhance

lipid visibility ultrastructurally within epidermal tissue, specimens were exposed to acrolein vapour (5% acrolein in PBS buffer pH 7.4) as the primary fixative for one hour in ice, and post-fixed with 0.5% ruthenium tetroxide with 0.25% potassium ferrocyanide for 1 h in a dark refrigerator. The specimens were then dehydrated by a series of alcohol and acetone rinses. Fresh Epon-Araldite mixture was then prepared with 10 mL Araldite 502, 12.5 mL Epon, 30 mL DDSA, and 1.3 mL DMP-39. The samples were placed in a two part acetone and one part Epon-Araldite mixture for 45 min, then 1:1 and 1:2 mixtures for 1 h each, then embedded in a mould and left to dry in a 37 °C oven for 20 h. Samples were sectioned at 90 nm and stained with either uranyl acetate and lead citrate or barium permanganate. This methodology has been modified from Fartasch *et al.*⁹

Evaluation of transmission electron microscopy

Each grid (with section) was viewed, evaluated and photographed on a transmission electron microscope (Hitachi 700, Twinsburg, OH, USA) operated at 75 kV. Images of stratum corneum and granulosum were taken digitally at $\times 5000$, $\times 25\,000$ and $\times 50\,000$, using a Peltier-cooled Tietz 2k $\times$ 2k charge coupled device camera (Gauting, Germany) mounted below the viewing chamber. Files were saved as Tagged Image Format Files. All evaluations were done by an investigator who was blinded to the source of the samples. Dogs were randomized but all images of one dog were evaluated together.

The upper and lower stratum corneum was evaluated. Emphasis was placed on the assessment of the intercellular spaces in the stratum corneum, the presence and location of LB and the morphology of the LL.

Statistics

Pre and post allergen exposure CADESI were compared using a mixed model analysis of variance (ANOVA). Unpaired and paired *t*-test were also used to compare scores across and within groups, respectively. A *P*-value less than 0.05 was considered significant.

Results

Canine atopic dermatitis and Extent Severity Index 03 scores

Analysis of variance showed a significant effect of time ($P = 0.04$) – with scores being higher after allergen exposure – and a significant group $\times$ time interaction ($P = 0.04$) – with atopic Beagles increasing their CADESI scores after allergen challenge and the normal dogs showing no significant change. An independent *t*-test showed no significant difference in the scores between the controls and the atopic Beagles before allergen exposure ($P = 0.95$) and significant differences after allergen exposure ($P < 0.01$) with the atopic dogs being higher than the controls. Within the atopic group, *t*-test showed a significant increase after allergen exposure ($P < 0.001$) while within the control group there were no significant changes ($P = 1.0$). In terms of distribution of lesions, in the atopic dogs, erythema (either diffuse or macular depending on the dog) developed on the face feet, ears and inguinal area.

Electron microscopy findings

In the images taken at $\times 5000$, at baseline (prior to allergen challenge), normal dogs showed a regularly organized and compacted stratum corneum (Figure 1a) while atopic dogs showed less compacted stratum corneum with wider intercellular spaces (Figure 1b). This finding was consistent and could be focally severe. Lamellar bodies were present intracellularly in the stratum granulosum in both normal and atopic dogs (images not shown). After

allergen exposure, normal dogs showed minimal to mild increase in intercellular spaces of the stratum corneum (Figure 1c) while atopic dogs developed focally severe widening of intercellular spaces (Figure 1d). Intercellular spaces were consistently filled with large amounts of lipid material in which LL were irregularly organized. Abnormal (e.g. amorphous and nonstructured) lipid accumulations were frequently present in the intercellular spaces in atopic samples.

In the images taken at $\times 25\,000$, prior to allergen exposure in normal dogs (Figure 2a), corneocytes were stacked parallel to each other and corneodesmosomes were easily identifiable while in the atopic dogs (Figure 2b) corneocytes showed disorganized arrangement. Intercellular spaces in atopic individuals were filled with abnormal lipid material and partially recognizable LL. After allergen exposure, mild widening of intercellular spaces could be observed in normal dogs (Figure 2c) while in atopic dogs (Figure 2d) marked disorganization of both corneocytes and lipid material was a common finding. Intercellular spaces were consistently filled with abundant and abnormal lipid material.

In the images taken at $\times 50\,000$, at baseline, organized and stacked LL were detected in normal dogs (Figure 3a) while delamination was a frequent finding in atopic samples (Figure 3b). After allergen exposure, LL were unchanged in normal dogs (Figure 3c) while they were greatly disorganized and separated by large amounts of lipids in atopic dogs (Figure 3d). The intercellular lipid was not organized into orderly LL and abnormal oval to globoid shaped LL were found. Another finding in atopic dogs, which was not seen in normal dogs, was the presence of LB inside corneocytes (Figure 4).

Discussion

In this study, numerous ultrastructural abnormalities were detected in the atopic dogs when compared to controls. These changes were focally severe and included disorganization of the LL, increased width between corneocytes, LB within corneocytes, and large amounts of amorphous intercellular lipids. The last change was more marked after allergen challenge.

Some of these changes are similar to previous reports describing irregularity and discontinuity of LL in atopic dogs with naturally occurring disease.¹⁵ A recent study also reported that the nonlesional skin of atopic dogs differed from the skin of healthy dogs in that the LL were highly disorganized.²⁰ In that study it was reported that the majority of intercellular spaces in atopic dogs appeared 'empty' and that there was a significant difference in the percentage of intercellular spaces filled by lipids between the normal and atopic dogs. In the present study, we also noticed disorganization of the LL, frequent lamellar delamination or 'roll-up' (see Figure 3b) as well as widening of LL. These changes have been described in human literature after injury or traumatic event to the skin.²¹ With a methodology that optimized lipid visibility, we were able to detect lipids in the intercellular spaces of atopic dogs which were not detected in a previous study.²⁰

The abnormalities found in this study are also in line with other reports in the human literature, in which LB

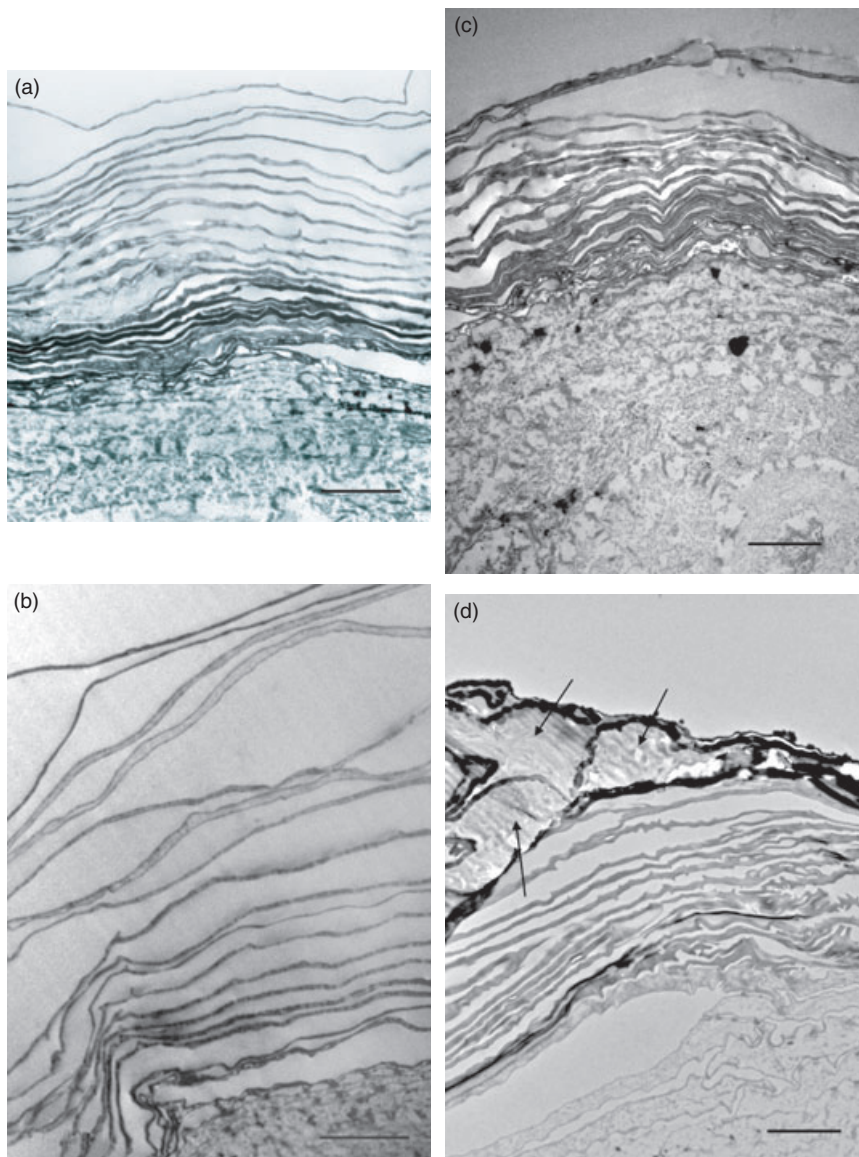


Figure 1. Electron micrographs of the upper epidermis before and after allergen exposure in control and atopic dogs. In normal dogs (a, c) the stratum corneum is well organized regardless of the exposure to the allergen (a = before allergen exposure, c = after allergen exposure). In atopic dogs wider intercellular spaces are noticeable (b, d), particularly after allergen exposure (d) when large amounts of lipids fill such spaces (arrows). Samples were post-fixed with 0.5% ruthenium tetroxide and 0.25% potassium ferrocyanide. Bar = 2.0 μ m.

within corneocytes were described.^{9,10} In our study also, LBs were found intracellularly in corneocytes. This finding was evident in nonlesional skin of atopic dogs in baseline biopsies. No intracellular LB was found in corneocytes of the control samples. It is accepted that in humans the presence of LBs in corneocytes is due to delayed extrusion.^{9,10} Whether the changes observed in these dogs are due to similar defect is unknown at this time. This is the first report of LBs in the corneocytes of atopic dogs. Whether this abnormality is limited to this colony of dogs or applies to dogs with naturally occurring AD is unknown.

This is also the first study that evaluated the effect of allergen exposure on the ultrastructure of the epidermis. Allergen challenge exacerbated the ultrastructural abnormalities of atopic dogs while normal dogs were unaffected. Focal widening of the intercellular spaces, release of lipid material, and disorganized LL were found. It is known that HDM possess enzymes that cause barrier impairment and increased allergen penetration²² but, in this study, these enzymes failed to alter the stratum corneum ultrastructure of normal dogs. Atopic skin, on

the contrary, being abnormal at baseline, was greatly affected by HDM exposure.

The effect of allergen challenge on lipid release is consistent with other studies in human medicine that have reported release of LB in large numbers subsequent to a variety of insults (e.g. excessive exposure to water,²¹ stress²³). Thus, keratinocytes react to an insult by releasing LBs in an attempt to restore barrier function and making more lipids available. However, in these atopic dogs the intercellular lipid was not organized into orderly LL and, after allergen challenge, abnormal oval to globoid shaped lamellae were found. The failure to form orderly LL may contribute to an ineffective barrier function in these atopic dogs. Transepidermal water loss has been shown to be increased in this model of canine AD.²⁴ The abnormalities identified in the ultrastructure of the stratum corneum could account for this. Additional studies are necessary to test this hypothesis.

Limited studies have been performed in atopic dogs to evaluate lipid abnormalities.^{25,26} A recent study documented decreased epidermal ceramides in atopic dogs compared to normal dogs.²⁵ Whether this decrease is

Figure 2. Electron micrographs of the stratum corneum before and after allergen exposure in control and atopic dogs. In normal dogs prior to allergen exposure (a), corneocytes are organized parallel to each other and corneodesmosomes are easily identifiable (white arrows). In atopic dogs (b) before allergen exposure, corneocytes show disorganized arrangement and the intercellular spaces are filled with abnormal lipid material (asterisks) and partially recognizable LL (arrows). After allergen exposure, minimal to mild widening of intercellular spaces is noted in normal dogs (c) while in atopic dogs (d) marked disorganization of corneocytes and LL (thin arrows) as well as wide intercellular spaces filled with abnormal lipid material (thick arrows) are a common finding. Samples were post-fixed with 0.5% ruthenium tetroxide and 0.25% potassium ferrocyanide. ICS = intercellular spaces. Bar = 0.5 μ m.

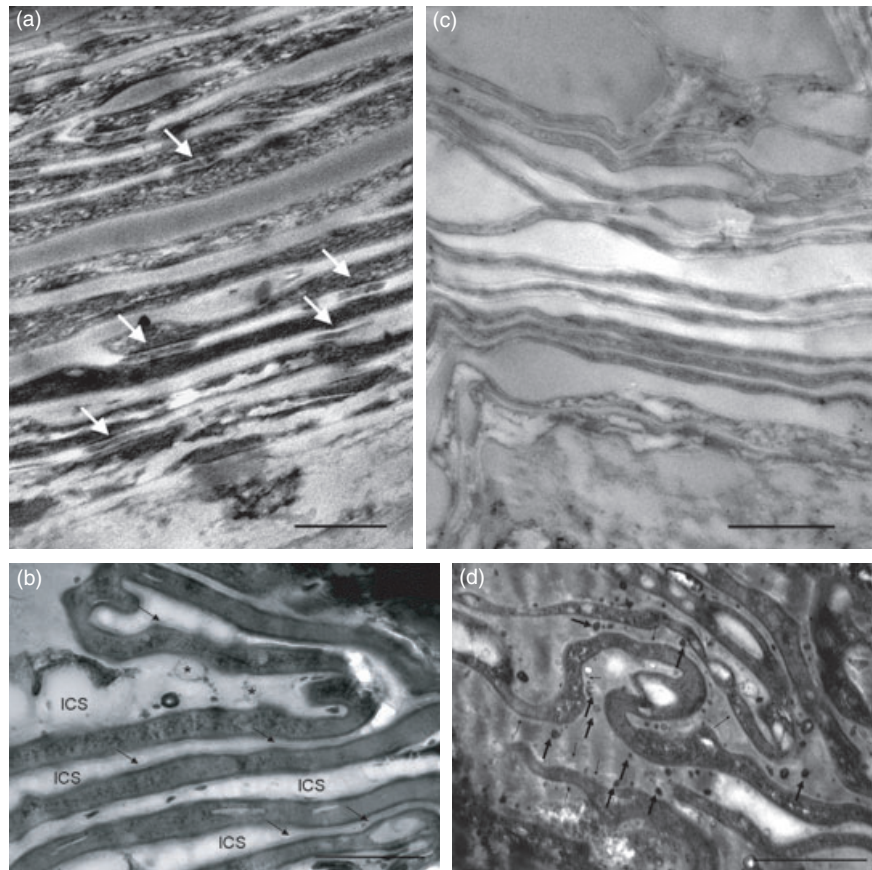
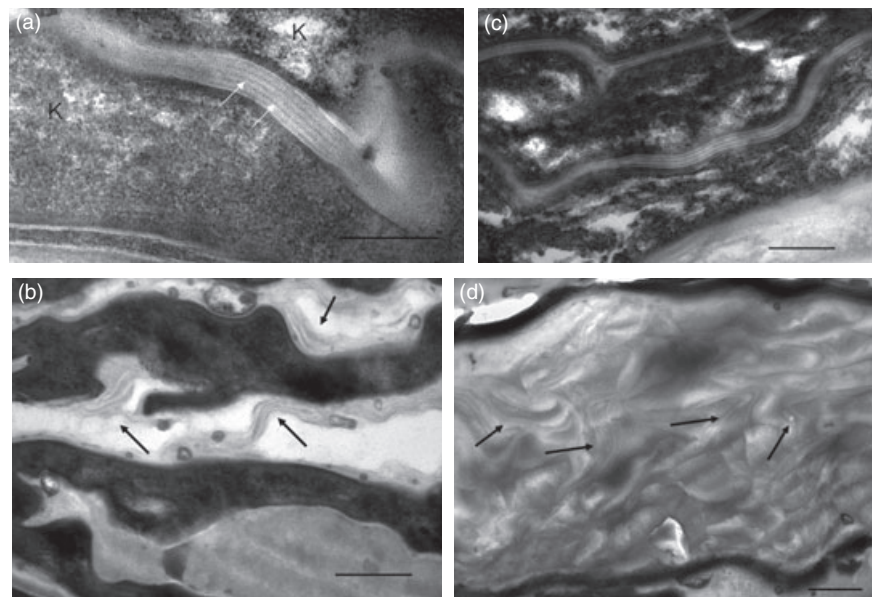


Figure 3. Electron micrographs of lipid lamellae before and after allergen exposure in control and atopic dogs. Before allergen exposure in normal dogs (a) normal lipid lamellae (white arrows) are easily detected while delamination is a frequent finding in atopic samples (b; black arrows). After allergen exposure, normal lipid lamellae are still evident in normal dogs (c) while in atopic dogs lipid lamellae are greatly disorganized (black arrows) and separated by large amounts of lipids in the intercellular spaces (d). Samples were post-fixed with 0.5% ruthenium tetroxide and 0.25% potassium ferrocyanide. K = keratinocyte; ICS = intercellular space. Bar = 200nm.



linked to an enzymatic abnormality as reported in human medicine²⁷ is unknown at this time. Clearly, additional research is needed before speculations can be made on how to treat such abnormalities. However, encouraging results have been recently reported in dogs with a preparation containing ceramides, free fatty acids, and cholesterol.²⁰ Unfortunately, that study did not report on clinical benefits associated with ultrastructural normalization.

Although studies on skin barrier function in canine AD are still in their infancy, it is crucial to start recognizing

that it is an important aspect of this disease and that further investigation is warranted. Whether the changes observed in the baseline biopsies are primary or secondary to prior sensitization is unknown. Nevertheless, the atopic dogs used in this study lived in a controlled environment where exposure to allergen outside of an experimental condition was tightly controlled. The sites used for baseline skin biopsies did not show any clinical evidence of inflammation and haematoxylin and eosin stains of those samples had not shown any evidence of

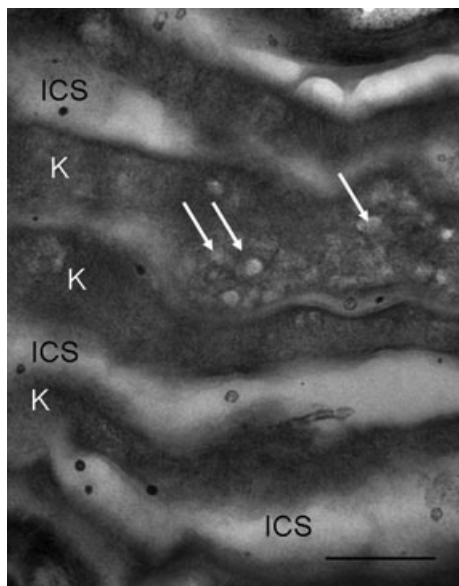


Figure 4. Electron micrograph of lamellar bodies (arrows) inside atopic keratinocytes. Sample was post-fixed with 0.5% ruthenium tetroxide and 0.25% potassium ferrocyanide. K = keratinocytes; ICS = intercellular spaces. Bar = 20 nm.

inflammatory infiltrate. These considerations would support the argument that the changes may be due to a primary perturbation in the skin barrier structure albeit in an experimental model with prior sensitization. Genetic studies are ultimately needed to address the question about primary abnormalities in this colony of dogs.

In human medicine, it is proposed that the classic paradigm of AD as primarily due to immunologic aberration ('inside/outside') needs to be reconsidered or shifted to include a primary defect in barrier function ('outside/inside') in the pathogenesis. It is proposed that such defects would lead to increased risk for allergic sensitization and development of an aberrant immunological response in individuals genetically predisposed to AD.²⁸ This shift in paradigm may lead to new treatments aimed at reducing the risk of allergic sensitization in predisposed patients and minimizing further skin damage in already clinically affected patients.²⁹ Future studies need to identify the biochemical abnormalities in this subset of atopic dogs to further validate this model of canine AD. This model could prove to be useful for a combined clinical and ultrastructural evaluation of treatments aimed at the restoration of the skin structure and barrier function, if these biochemical changes prove similar to those of naturally occurring atopic dogs.

In conclusion, this experimental model of canine AD has baseline ultrastructural epidermal changes similar to the ones reported in atopic dogs with naturally occurring disease and in human patients with AD. These changes were exacerbated in the atopic dogs by allergen challenge and the resulting flare-up of dermatitis.

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Résumé Un défaut de la fonction barrière cutanée a été supposé dans la dermatite atopique canine. Dans cette étude prospective, contrôlée, l'ultrastructure des couches épidermiques superficielles a été étudiée en utilisant un modèle expérimental de dermatite atopique canine. Sept beagles atopiques sensibilisés à *Dermaphagoides farinae* et quatre beagles sains ont été utilisés comme contrôles. Les chiens normaux et atopiques ont été soumis à *D. farinae* pendant 3 jours. Les signes cliniques ont été évalués et des biopsies cutanées ont été réalisées en zone inguinale avant et 3 jours après l'exposition allergénique. Les échantillons ont été traités afin d'améliorer la visibilité des lipides et analysés par microscopie électronique à transmission. L'accent a été mis sur l'étude des lamelles lipidiques (LL) et des corps lamellaires (LB) du stratum corneum. Après provocation allergénique, les beagles atopiques ont développé une dermatite sévèrement prurigineuse alors qu'aucune lésion n'était notée chez les chiens contrôles. Avant l'exposition allergénique, d'un point de vue ultrastructural, les beagles atopiques montraient focalement de sévères anomalies d'organisation des LL et des espaces intercellulaires, plus larges, contenaient un matériel lipidique anormal. Chez les beagles atopiques, les LB étaient fréquemment à l'intérieur des cornéocytes, contrairement aux chiens contrôles. Après provocation allergénique, une augmentation plus marquée des espaces intercellulaires a été observée au niveau du stratum corneum des beagles atopiques tandis qu'aucun changement n'était observable chez les chiens normaux. Les espaces intercellulaires des beagles atopiques étaient remplis d'amas abondants d'un matériel lipidique anormal et de LL hautement désorganisés. Il a ainsi été conclu que des différences de base de l'ultrastructure de la peau existe entre les beagles normaux et les beagles atopiques sensibilisés expérimentalement et que ces changements sont aggravés par une provocation allergénique ce qui résulte en une dermatite. Ultrastructuralement, avant le défi d'allergènes, Beagles atopiques affichent focalement graves anomalies dans LL organisation et les plus larges intercellulaires espaces contenant des matériaux de lipides anormale. Dans Beagles atopiques, LB fréquemment trouvées à l'intérieur cornéocytes alors que cette conclusion n'était pas respectée dans les contrôles. Après le défi d'allergènes, nouvelle augmentation des espaces intercellulaires a été observée dans le stratum corneum de Beagles atopiques alors qu'aucune modifications sensibles ont été observées dans les chiens normales. Espaces intercellulaires en Beagles atopiques ont été remplis avec des montants abondantes de matériel de lipides anormale et LL hautement désorganisée. Il est conclu que les différences de configuration de référence dans l'ultrastructure de la peau existent entre la normale et sensibilisés expérimentalement atopiques Beagles et que ces modifications sont aggravées par défi allergènes et l'accès résultante de la maladie de Dermatite.

Resumen Se ha propuesto la hipótesis de que una anomalía en la función de barrera de la piel está implicada en el desarrollo de dermatitis atópica canina (AD). En este estudio prospectivo controlado se investigó la ultraestructura de los estratos superiores de la piel utilizando un modelo experimental de AD. Se incluyeron siete perros atópicos de raza Beagle sensibilizados frente a *Dermaphagoides farinae* y cuatro perros Beagle sanos fueron utilizados como controles. Tanto los perros normales como los atópicos fueron expuestos a *D. farinae* durante 3 días. Se valoraron los signos clínicos y se tomaron biopsias de piel de la zona inguinal antes y tres días tras la exposición al alérgeno. Las muestras fueron procesadas para incrementar la visibilidad de los lípidos y se evaluaron mediante microscopía electrónica de transmisión. Se puso más énfasis en la evaluación de las lamelas lipídicas (LL), cuerpos lamelares (LB) y el estrato corneo. Tras la exposición al alérgeno, los perros Beagle atópicos desarrollaron dermatitis pruriginosa severa, mientras que no se observaron lesiones en los perros control. Ultraestructuralmente antes de la exposición al alérgeno, los perros Beagle atópicos mostraron anomalías severas en la organización de las LL y más amplios espacios intercelulares que contenían material lipídico anormal. En perros Beagle atópicos los LBs estaban con frecuencia dentro de corneocitos, algo que no se observó en perros control. Tras la exposición al alérgeno se observó un mayor incremento en los espacios intercelulares en el estrato corneo de perros atópicos mientras que no hubo cambios apreciables en los perros normales. Los espacios intercelulares en los perros atópicos se rellenaron con abundante material lipídico anormal y LL desorganizadas. Se concluye que existen diferencias basales en la ultraestructura de la piel entre perros normales y perros atópicos Beagle sensibilizados de forma experimental, y que estos cambios son agravados tras la exposición al alérgeno y la dermatitis resultante.

Zusammenfassung Es wird hypothesisiert, dass bei der caninen atopischen Dermatitis (AD) einer Funktionsschwäche der Hautbarriere besteht. In dieser prospektiven, kontrollierten Studie wurde die

Ultrastruktur der oberen epidermalen Schichten mit Hilfe eines experimentellen Modells der caninen AD untersucht. Es wurden sieben atopische Beagles, die gegen *Dermatophagoides farinae* sensibilisiert worden waren und vier gesunde Beagles als Kontrolltiere verwendet. Sowohl normale als auch atopische Hunde wurden mit *D. farinae* 3 Tage lang provoziert. Die klinischen Symptome wurden bewertet und vor und drei Tage nach der Exposition zu den Allergenen von der Inguinalgegend Hautbiopsien entnommen. Die Proben wurden bearbeitet, um die Sichtbarkeit der Lipide zu verbessern und mittels Transmissionselektronenmikroskop untersucht. Ein Schwerpunkt war die Untersuchung der Lipidlamellen (LL) und der lamellären Körper (LB) des Stratum corneums.

Nach der Provokation mit Allergen entstand bei den atopischen Beagles eine hochgradige juckende Dermatitis, während bei den Kontrolltieren keine Hautveränderungen bemerkt wurden. Ultrastrukturell zeigten die atopischen Beagles vor der Provokation mit Allergenen fokale hochgradige Abweichungen der Organisation der LL und erweiterte Interzellularspalten, die abnormales Fettmaterial enthielten. Bei den atopischen Beagles wurden die LB häufig innerhalb der Korneozyten gefunden, was bei den Kontrolltieren nicht der Fall war. Nach der Provokation mit Allergenen wurde eine weitere Vergrößerung der Interzellularspalten im Stratum corneum der atopischen Beagles beobachtet, während keine merklichen Veränderungen bei den normalen Hunden gesehen wurden. Die Interzellularspalten der atopischen Beagles waren mit reichlichen Mengen an abnormalem Fettmaterial und ungeordneten LL gefüllt. Es wird daraus geschlossen, dass bei normalen und experimentell sensibilisierten atopischen Beagles bereits unterschiedliche Ausgangswerte im Bezug auf die Ultrastruktur der Haut bestehen und dass diese Veränderungen durch eine Provokation mit Allergenen und den daraus resultierenden Dermatitis Schüben verstärkt werden.