

Immunohistochemical evaluation of filaggrin polyclonal antibody in atopic and normal beagles

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Conflict of Interest

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antibody used with other epidermal proteins besides
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Abstract

In human atopic dermatitis (AD), impairments in skin barrier function are emphasized and hypothesized to increase risk of allergic sensitization. Filaggrins, crucial proteins for keratinization, are decreased in lesional and nonlesional human atopic skin. As canine AD shares numerous similarities with the human counterpart, this study aimed to evaluate a polyclonal antibody against human filaggrin in atopic beagles sensitized to house dust mites (HDM) and normal healthy dogs. The effects of HDM exposure on immunostaining and clinical signs were evaluated in both groups. Positive immunohistochemical staining with anti-filaggrin antibody was evaluated both objectively and subjectively by two blinded investigators. Pearson correlation test showed significant correlation between objective and subjective scores, both at baseline and after allergen exposure ($r = 0.80$; $P = 0.0017$ and $r = 0.75$; $P = 0.013$ respectively). Analysis of variance showed significant effect of time ($P = 0.01$) with immunostaining being higher in baseline samples than after HDM exposure. It also showed a significant group $\times$ time interaction ($P = 0.02$) with immunostaining not changing significantly over time in atopic dogs, while decreasing in normal dogs after HDM exposure. An independent t -test showed that, at baseline, atopic beagles had significantly less positive immunostaining than controls ($P = 0.009$) and that, after HDM exposure, there was no significant difference between groups. No correlation existed between clinical scores and immunostaining. In atopic dogs immunostaining was characterized by faint granular staining, while normal samples showed discrete intense staining. Moreover, immunostaining was present in all epidermal layers in many samples, suggesting cross-reactivity of the

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 Most of the research on canine AD has focused on the aberrant immunological responses^{3,4} and minimal information exists on the barrier function of the skin in this condition.⁵

Impairment of the skin barrier has been extensively demonstrated in humans with AD and it is known that a genetically impaired skin barrier function is present in both lesional and nonlesional skin.^{6,7} A disturbed differentiation, including changes in lipid and protein composition and content, have been demonstrated to cause an impaired skin barrier function and it is hypothesized that this defective permeability allows an enhanced penetration of environmental allergens, thus increasing the risk of sensitization in genetically predisposed individuals.^{8,9} As barrier function is negatively affected by Th2 cytokines,^{10,11} once inflammation is triggered, further impairment of the skin barrier develops leading to a self-perpetuating cycle of additional sensitizations and progressive worsening of the disease.

Filaggrins (filament-aggregating proteins) are complex polypeptides that play a crucial role in keratinization and in the barrier function of the skin.¹² The precursor of filaggrin is profilaggrin. It is contained in keratohyaline granules, an essential component of the upper layers of the epidermis. Upon terminal differentiation of granular cells, profilaggrin is proteolytically cleaved into filaggrin peptides, which rapidly aggregates the keratin cytoskeleton, causing the collapse of the granular cells into flattened anuclear squames.¹² Humans with AD have decreased expression of filaggrin in both lesional and nonlesional skin.¹³ It is estimated that more than half of the children with moderate-to-severe AD carry loss-of-function filaggrin mutations.^{14–16} Since the first study reporting the two loss-of-function mutations (R501X and 2282del4) in the filaggrin gene, more studies have replicated these findings and reported other mutations in various populations.^{17–19} Interestingly, associations of the filaggrin

mutations were found particularly with the extrinsic subtype of AD, disease characterized by high total serum IgE levels and concomitant allergic sensitizations.²⁰ This association has suggested that disturbances of the epidermal barrier caused by filaggrin variants facilitate allergen penetration into the subepidermal layers and, depending on the cytokine environment, would lead to allergic sensitization. Despite the importance of the filaggrin mutations and their demonstrated role as a strong risk factor for AD, not all patients with AD suffer from these mutations. Importantly, filaggrin synthesis can be modulated and Th2 cytokines are known to suppress it.²¹ Thus, filaggrin deficiency can also develop in individuals that do not have a mutation in the filaggrin gene.

Canine AD shares many similarities with the human counterpart both clinically and immunologically²² and it is known that the epicutaneous route of allergen exposure plays an important role in the canine disease.²³ However, minimal information exists on the barrier function of canine epidermis and the role of filaggrin in canine AD is unknown.

Many of these questions would be very 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 also greatly affected by environmental conditions (temperature and humidity), dietary intake, stage of the disease and topical therapy (type and frequency). Thus, it would be very difficult to control for all these factors using dogs with naturally occurring AD. An experimental model for canine AD, in which lesions can be directly induced and monitored and where environmental and dietary conditions are strictly controlled, has been identified.^{23,24} In this model high-IgE beagles consistently develop skin lesions and pruritus upon environmental exposure to an allergen of reference (house dust mite, HDM). The advantages of having access to this model for this preliminary investigation are multiple. Besides being able to control for external factors that may affect barrier function, using research dogs allows the possibility of controlled allergen challenge and the collection of multiple biopsies that may not sometimes be allowed in privately owned atopic dogs. Derangements of the barrier function could be evaluated in a sequential manner upon allergen challenge to determine whether alterations are primary or secondary to inflammatory changes induced by allergen challenge or both. Epidermal changes could then be correlated with severity of clinical signs to evaluate clinical implications of barrier impairment. In the past, unsuccessful attempts had been made to reproduce clinical disease by simply selecting for high-IgE responders;²⁵ thus, it is conceivable that the dogs in the aforementioned experimental model have other important abnormalities, besides high IgE production, that make them a suitable model and, possibly, close to the naturally occurring disease. In this study it was hypothesized that, similar to what is reported in human AD, these dogs have an impairment of the skin barrier function that makes them more prone to allergen penetration. Due to their immunological make-up, once allergen is absorbed, allergic sensitization ensues.

The purpose of this study was to evaluate a polyclonal antibody specific against human filaggrin in beagles with experimentally induced AD and compare staining patterns in skin samples of normal beagles. Subsequently, immunostaining was evaluated in both groups after exposure with HDM. It was hypothesized that decreased immunostaining exists in atopic beagles in nonlesional skin and prior to allergen challenge and that immunostaining would be further decreased after allergen exposure.

Materials and methods

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

Animals

Eleven atopic beagles and four healthy beagles (normal controls) were used for this study. The high-IgE beagles had been previously epicutaneously sensitized to HDM and were known to develop skin lesions and pruritus upon allergen exposure to HDM.^{23,24} Previous biopsies of these lesions have demonstrated that they are histologically and immunologically compatible with naturally occurring canine AD.²⁴

The healthy controls were selected to match as close as possible the age and sex of the atopic 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 environmental challenge with HDM (pilot studies, results not shown here) and negative serology testing for allergen-specific IgE. The control dogs were obtained from another research colony housed at the same institution.

Allergen challenge

All beagles were environmentally challenged with HDM, as previously described.^{23,24} Briefly, HDM was applied daily for 3 days on the floor of a plastic airline transport kennel. Each dog was placed in the kennel for 3 h each day, for three consecutive days. Kennels were washed at the end of the challenge. The concentration and volume of HDM used were selected based on previous studies.^{23,24} To control for potential effects of HDM on filaggrin expression, even in the absence of an allergic reaction, the healthy beagles were similarly challenged.

Clinical evaluation

All dogs were evaluated and scored before and 6 h after each exposure and daily thereafter for 5 days. Clinical signs were evaluated using a validated scoring system (Canine Atopic Dermatitis and Extent Severity Index 03, CADESI).²⁶ 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 in the statistical 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 symptom.

Skin biopsies

Two 6-mm biopsy specimens were taken from each dog before allergen challenge (nonlesional area) and 3 days after challenge (lesional area in sensitized 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, based on previous studies, this is an area that consistently shows lesions after allergen challenge.^{23,24} The 3-day time frame was selected based on previous studies as both skin lesions and pruritus reach their peak on the third day of the experiment.²⁴ Biopsies were taken after local anaesthesia with lidocaine and sutured routinely.

Immunohistochemistry: processing of the samples

The suitability of commercially available anti-filaggrin antibodies (both monoclonal and polyclonal) on canine tissue was evaluated in pilot studies (data not shown).¹³ For the present study, the following methodology was implemented. Samples were fixed in 10% buffered neutral formalin for 24 h and then transferred into phosphate-buffered saline (PBS), dehydrated and embedded in paraffin. Sections were cut at 5 µm and placed on SuperFrost positively charged slides (Erie Scientific Company, Portsmouth, NH, USA). Sections were deparaffinized with xylene, rehydrated with PBS, quenched with 0.3% hydrogen peroxide and blocked first with 5% normal goat serum and subsequently with 2.5% normal horse serum. The primary antibody used was a rabbit polyclonal against filaggrin (Abcam, Cambridge, MA, USA). The detection method used was a horseradish peroxidase-streptavidin kit (Histostain Plus; Zymed Laboratories Inc., San Francisco, CA, USA). Control slides were prepared without the primary antibody.

Immunohistochemistry: imaging and image analysis

Slides were examined microscopically (Nikon Labophot microscope, Melville, NY, USA) and digital photographs were taken at ×250 and ×400 magnification with a Nikon Coolpix 990 camera (Melville, NY, USA). A total of six photographs were taken of each section and files were saved as Tagged Image Format files.²⁷

The ×250 photos were used to subjectively score the amount of filaggrin present in the stratum granulosum. Photographs were evaluated by two blinded investigators who scored both the extent and intensity of staining in the stratum granulosum of each picture on a scale of 0–3 (0 = absent and 3 = severe). The score of extent and intensity of staining were added and the mean of the total scores were calculated and used for analysis.

The ×400 photographs were used to calculate the amount of immunostaining present in each section. One examiner opened each photograph in ImageJ (<http://rsb.info.nih.gov/ij/>) and a pixel intensity threshold was determined to include only those image pixels in immunopositive areas. The epidermis was traced and the total pixel count of immunopositive area was calculated. The percentage of immunostaining area was calculated by dividing the count of the immunopositive areas by the area of the epidermis.²⁷

Statistics

Pre- and post-allergen exposure CADESI and positive immunostaining scores were examined separately and compared using a mixed model ANOVAs. The per cent of immunostaining of normal and atopic beagles was also compared using *t*-tests. Relationships among these measurements were evaluated using Pearson product-moment correlations. A value of $P < 0.05$ was considered statistically significant.

Results

Animals

The atopic beagle group was composed of six intact males and five intact females, who were 2 years old at the time of the study. The healthy control group was composed of three intact females and one intact male, ranging from 2 to 3 years of age.

CADESI scores

In the atopic dogs, erythema (either diffuse or macular depending on the dog) developed on the face, feet, ears and inguinal area. An analysis of variance showed a significant effect of time ($P = 0.04$) with clinical scores being higher after allergen exposure. Additionally, ANOVA showed a significant group × time interaction ($P = 0.04$) with atopic beagles having higher CADESI scores after allergen challenge while normal dogs did not show a

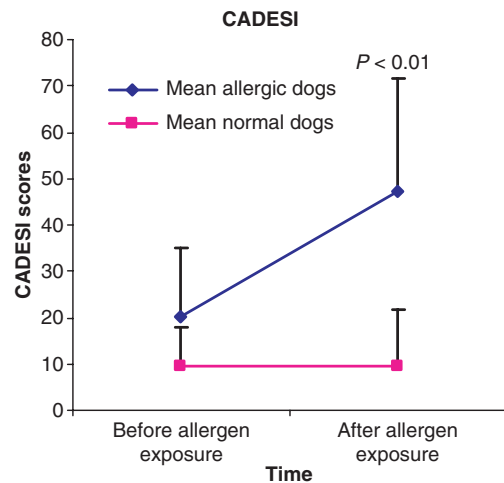


Figure 1. Mean clinical scores (canine atopic dermatitis extent and severity index, CADESI) for both normal and atopic beagles before and after allergen exposure. An independent *t*-test showed no significant difference in the scores between the controls and the atopic beagles before allergen exposure ($P = 0.95$) and significant differences after allergen exposure ($P < 0.01$) with the atopic dogs being higher than in the controls.

significant change (Figure 1). An independent *t*-test showed no significant difference in the scores between the controls and the atopic beagles before allergen exposure ($P = 0.95$). After allergen exposure atopic dogs had significantly higher scores ($P < 0.01$) when compared with controls. Within the atopic group, *t*-test showed a significant increase after allergen exposure ($P < .001$), while within the control group there were no significant changes ($P = 1.0$).

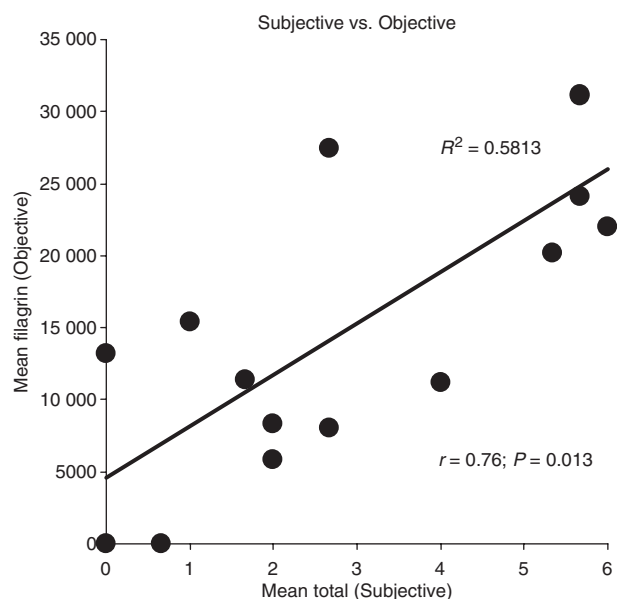


Figure 2. Scatter plot showing the correlation between subjective scores and objective measurements of the total number of pixels of immunopositive areas stained after allergen exposure when both normal and atopic dogs were combined. Several atopic dogs had no positive results and scored 0.

Correlation between subjective scores and objective (semi-quantitative) measurements of positive immunostaining

Pearson correlation test showed a significant correlation between objective and subjective scores ($r = 0.80$; $P = 0.0017$) at baseline and after allergen exposure ($r = 0.76$; $P = 0.013$, Figure 2). Thus, for the remainder of the analysis only the objective measurements were used.

Immunostaining in normal versus atopic dogs and modulation by HDM exposure

In many samples, immunostaining was evident in the stratum granulosum as well as in lower epidermal layers. Positive immunostaining in the stratum granulosum appeared different between groups. In samples of normal skin (Figure 3a,b, pre- and post-allergen exposure respectively) discrete granules with intense staining were observed. In skin samples from atopic dogs (Figure 4a,b, pre- and post-allergen exposure respectively) a very fine, granular fainter staining was evident. Faint staining of vessels was also noted in some samples.

An analysis of variance showed a significant effect of time ($P = 0.01$) with immunostaining being higher in baseline samples than after allergen exposure. Additionally,

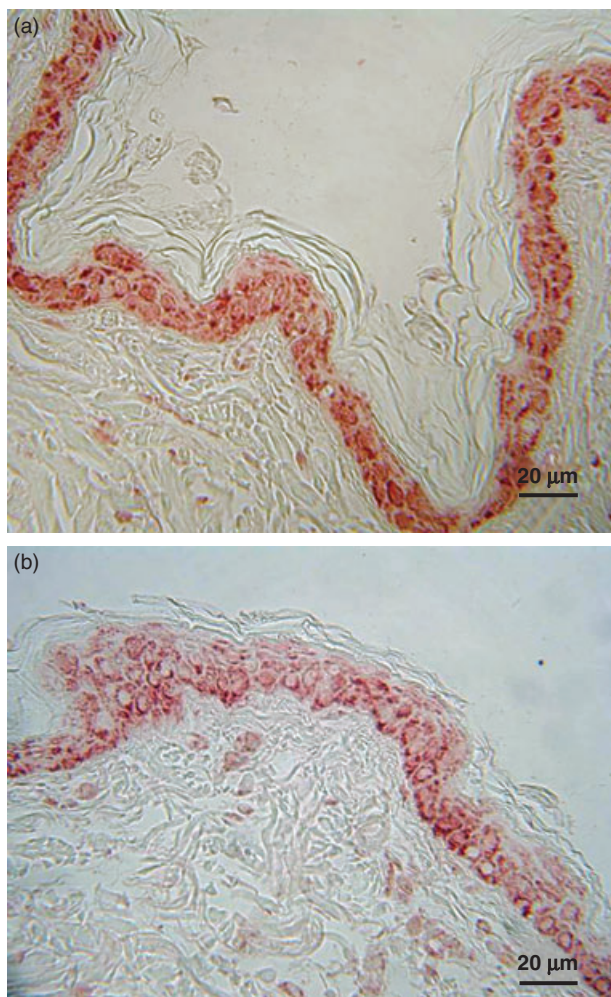


Figure 3. Immunohistochemical stain of normal epidermis (a) pre- and (b) post-allergen challenge using a polyclonal antibody against filaggrin. Note the discrete granular pattern in the stratum granulosum. Bar = 20 µm.

ANOVA showed a group $\times$ time interaction ($P = 0.02$) with immunostaining in atopic dogs not changing significantly over time, while a significant decrease was seen in normal dogs after HDM exposure. Based on an independent t -test, at baseline, atopic beagles had significantly less positive immunostaining at baseline, when compared with normal controls ($P = 0.009$). However, after HDM exposure, there was no significant difference between groups (see Figure 5). No significant correlation was found between positive immunostaining and CADESI scores.

Discussion

The current study evaluated staining patterns of a cross-reactive, anti-human filaggrin-specific polyclonal antibody in the skin of atopic and healthy beagles. Decreased positive immunostaining was observed in an experimental model of canine AD when compared with healthy beagles. Interestingly, exposure to HDM decreased the positive immunostaining and this decrease was particularly evident in normal dogs.

The positive immunostaining observed with this cross-reacting anti-human filaggrin antibody suggests cross-reactivity with other canine epidermal proteins, as various levels of the epidermis revealed positive immunostaining, whereas in human skin the positive staining is limited to the stratum granulosum and stratum corneum.

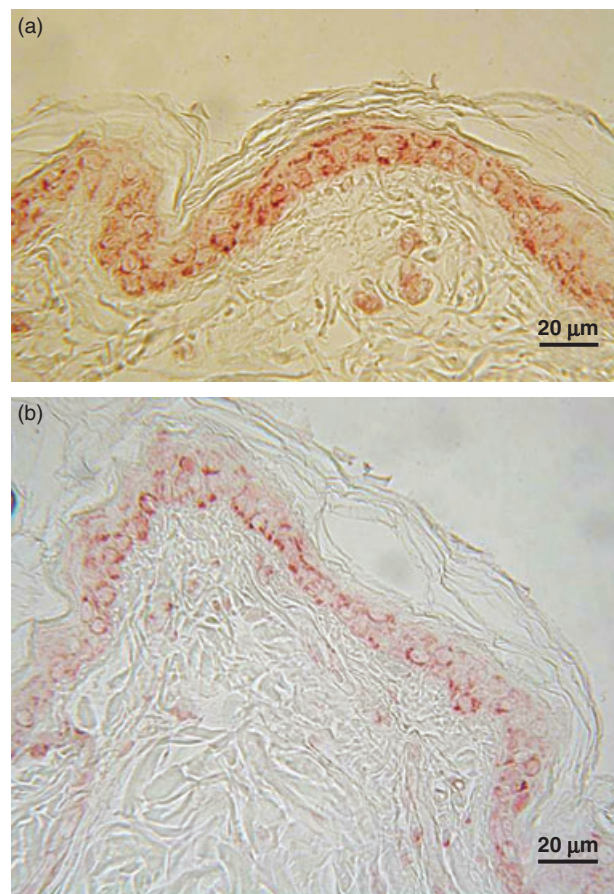


Figure 4. Immunohistochemical stain of atopic epidermis (a) pre- and (b) post-allergen challenge using a polyclonal antibody against filaggrin. Note the fine granular pattern in the stratum granulosum. Bar = 20 µm.

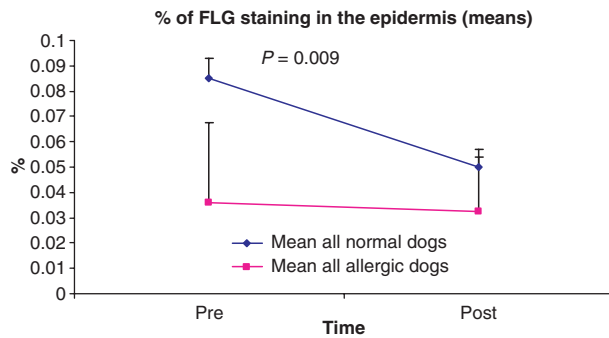


Figure 5. Mean values and standard deviations of percentage of immunopositive pixels in the epidermis of normal and atopic dogs before and after allergen challenge. An independent *t*-test showed that, at baseline, atopic beagles had significantly less immunostaining than normal controls ($P = 0.009$) and that, after house dust mite exposure, there was no significant difference between groups.

Cross-reactivity has occasionally been observed in human medicine, when various monoclonal antibodies against filaggrin were used.²⁸ Comparing seven different monoclonal antibodies against human epidermal filaggrin, it was evident that some do not recognize profilaggrin (e.g. stained the corneocyte matrix but not the keratohyaline granules), while others (AHF4-6) stained the cytoplasm of basal cells.²⁸ Subsequently, it was shown that these antibodies (AHF5 and AHF6) cross-react with cytokeratins. It was concluded that filaggrin and cytokeratins share several antigenic determinants and that filaggrin bears at least one epitope absent from its precursor.

Filaggrins are a heterogeneous group of proteins with different sizes, charges and sequences, thus represent a group of proteins rather than one individual protein. In pilot studies, all commercially available polyclonal and monoclonal antibodies for both human and mouse filaggrin and profilaggrin were evaluated for their reactivity in canine skin. Some monoclonal antibodies failed to cross-react, while others, which gave a nice band of staining in the stratum granulosum and corneum of human skin, revealed a staining pattern similar to the pattern observed with the polyclonal antibody used in this study. However, the staining was less intense than with the polyclonal antibody. By using the polyclonal antibody, the sensitivity was maximized and therefore resulted in a useful, measurable reaction due to the fact that several available (presenting) epitopes were identified. As with all polyclonal antibodies reactions, specificity is sacrificed to some point and expanded reactions can result in the staining of false positives. The latter has occasionally been observed with monoclonal antibodies as well.²⁸ Subsequent to performance of this study, the authors have stained human skin with the polyclonal antibody used for this study and, similar to the results in the dogs, various levels in the epidermis revealed positive staining. Thus, the pattern of staining is most likely due to the reported cross-reactivity with other epidermal proteins, such as cytokeratins.

The reason for the difference in staining patterns observed in atopic versus normal dogs is not known at this point of time. Large discrete granular intense staining was consistently seen in normal dogs, while atopic dogs revealed finer and faintly stained granules. Similar observations were not reported in the human literature.¹³

It could be speculated that chemical characteristics of these proteins may differ between atopics and normal dogs, similar to the way that different melanins and size of melanosomes exist between individuals with dark or fair skin.²⁹ Additional immunocytochemical studies would be necessary to investigate this hypothesis. Alternatively, atopic beagles may have an altered or accelerated keratinization. Increased desquamation has been well documented in humans with AD,³⁰ but it is unknown whether this occurs in dogs.

The decreased positive immunostaining in nonlesional canine atopic skin reflects the situation in humans.¹³ The decreased staining in nonlesional atopic skin does not necessarily imply a genetic mutation of filaggrin. Upon allergen exposure, a Th2 polarization develops in acute patch test lesions in these dogs.³¹ Alternatively, the synthesis of proteins such as filaggrins could be modulated by a release of Th2 cytokines.²¹ Whether these dogs have a higher expression of Th2 cytokines in nonlesional skin when compared with normal controls has not been determined. It is also unknown whether these dogs have any filaggrin mutation.

Various scoring systems have been documented for the evaluation of immunohistochemistry and the authors were interested in evaluating how a subjective score would relate to a measurement provided by imaging software. A very strong correlation between subjective and semi-quantitative, objective measurements were seen in this study. However, it is important to note that the calibration of the threshold and the tracing could introduce some variability and error. To minimize additional factors that could increase variability, all samples were run at the same time with the same lot of antibody.

The decreased positive immunostaining after HDM exposure in nonallergic dogs is not surprising considering the proteolytic properties of HDM.³²⁻³⁵ The proteolytic activity of allergens has been considered an important factor in the pathogenesis of allergies; by disruption of tight junctions the passage of their own and other allergens is facilitated across the epithelium.³⁶ The proteolytic activity may furthermore alter the skin barrier function in a dose- and time-dependent manner. This has been shown with Der f 1, one of the major human allergens of *Dermaphagoides farinae*.³⁷

Alternatively, HDM could modulate proteins such as filaggrin by altering intracellular Ca^{2+} ,³⁸ which in turn has an effect on keratinization.^{39,40} This action is independent on any allergic response. The lack of a significant decrease in immunostaining in the atopic dogs may be partly explained by the fact that expression was much lower at baseline, making detection of significant changes more difficult to appreciate.

Limitations of the current study include challenges associated with using non-species-specific reagents, and the small number of dogs evaluated. A larger population would assist to decrease variability and increase power. However, this small number of samples is not dissimilar to a immunohistochemical study reported in the human literature using antibodies against filaggrin¹³ in which significant differences between populations were documented.

In conclusion, immunostaining using a polyclonal antibody against human filaggrin showed a significant

decrease in immunoreactivity in atopic beagles compared with normal beagles and an additional decrease following HDM exposure. Further studies are required to evaluate the specificity of this anti-human filaggrin antibody for canine filaggrin, as differences in staining patterns suggest a possible cross-reactivity with other intraepidermal proteins in dogs.

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Résumé Dans la dermatite atopique humaine (AD), les défauts de la fonction barrière cutanée seraient responsables d'une augmentation des risques de sensibilisation aux allergies. Les filaggrines, protéines essentielles de la kératinisation, sont diminuées dans la peau lésionnelle et non-lésionnelle des hommes atopiques. Comme la dermatite atopique canine partage de nombreux points communs avec son équivalent humain, cet essai a pour but d'étudier les anticorps polyclonaux dirigés contre la filaggrine chez des beagles sains et atopiques sensibilisés aux acariens de la poussière de maison (HDM). Les effets de l'exposition aux acariens de la poussière de maison sur l'immunomarquage et les signes cliniques ont été évalués dans les deux groupes. Les marquages immunohistochimiques à l'aide d'anticorps anti-filaggrine ont été évalués objectivement et subjectivement par deux investigateurs en aveugle. Le test de Pearson a révélé une corrélation significative entre les scores objectifs et subjectifs avant et après exposition allergénique ($r = 0.80$; $P = 0.0017$; and $r = 0.75$; $P = 0.013$, respectivement). L'analyse de la variance a montré un effet significatif du temps ($P = 0.01$) avec des immunomarquages plus élevés dans les échantillons de référence qu'après exposition aux acariens. Il a également été montré que l'interaction groupe x temps ($P = 0.02$) avec immunomarquage ne changeait pas significativement à travers le temps chez les chiens atopiques alors qu'elle diminuait chez les chiens normaux après exposition aux HDM. Un test t indépendant a montré qu'avant exposition allergénique, les beagles atopiques avaient un immunomarquage significativement moins positifs que le groupe contrôle ($P = 0.009$) et que, après exposition, il n'y avait pas de différence significative entre les groupes. Aucune corrélation n'existait entre scores cliniques et immunomarquages. Les immunomarquages des chiens atopiques étaient caractérisés par une coloration granuleuse pâle tandis que les échantillons des chiens normaux présentaient une coloration intense lisse. En outre, un immunomarquage était présent dans toutes les couches épidermiques de plusieurs prélèvements suggérant une réactivité croisée de l'anticorps utilisé avec d'autres protéines épidermiques que la filaggrine.

Resumen En la dermatitis atópica humana, se enfatizan las irregularidades en la función de barrera de la piel con la hipótesis de que aumentan el riesgo a la sensibilidad alérgica. Las filaggrinas, proteínas de importancia crucial en la queratinización, están disminuidas en la piel de humanos con atopía en zonas de lesión y sin lesión. Ya que la dermatitis atópica canina comparte numerosas similitudes con la forma humana. Este estudio está enfocado a evaluar la utilización de un anticuerpo policlonal frente a la filaggrina humana en perros atópicos sensibilizados a ácaros del polvo del hogar, y en perros normales sanos. Los efectos de la exposición al ácaro del polvo del hogar en la tinción por inmunohistoquímica en los signos clínicos se evaluaron en ambos grupos. Se evaluó la positividad a inmunohistoquímica con el anticuerpo frente a la filaggrina por dos investigadores independientes de forma objetiva y subjetiva. Se utilizó una prueba de correlación de Pearson la cual nuestro una correlación significativa entre los valores objetivos y subjetivos, tanto a nivel basal como tras la exposición al alérgeno ($r = 0.80$; $P = 0.0017$; y $r = 0.75$; $P = 0.013$, respectivamente). El análisis de varianza mostró un efecto significativo del tiempo ($P = 0.01$) con la positividad a inmunohistoquímica siendo mayor en las muestras basales que tras la exposición al alérgeno. También mostró una interacción significativa entre el grupo x tiempo, con la positividad a inmunohistoquímica sin un cambio significativo a lo largo del tiempo en los perros atópicos, mientras que decrecía en los perros normales tras la exposición al ácaro del polvo del hogar. Una prueba t independiente mostró que, a nivel basal, los perros Beagle atópicos tenían significativamente menor positividad con la inmunohistoquímica que los perros control ($P = 0.009$), y que, tras la exposición al ácaro del polvo del hogar, no había diferencias significativas entre los grupos. No se observó correlación entre los valores clínicos y la positividad a inmunohistoquímica. En los perros atópicos la inmunohistoquímica se caracterizó por una tinción pálida granular, mientras que las muestras normales mostraron una positividad discreta e intensa. Además, la positividad a inmunohistoquímica se presentó en todos los estratos epidérmicos en muchas muestras, lo cual sugiere una reactividad cruzada del anticuerpo utilizado con otras proteínas epidérmicas además de la filaggrina.

Zusammenfassung Bei der atopischen Dermatitis (AD) des Menschen spielen Mängel bei der Funktion der Hautbarriere eine Rolle und es wird hypothesisiert, dass sie das Risiko einer allergischen Sensibilisierung erhöhen. Filaggrine, wichtige Proteine bei der Keratinisierung, sind in lässionaler und nicht-lässionaler atopischer Haut des Menschen vermindert. Da die canine AD zahlreiche Ähnlichkeiten mit der humanen Form aufweist, zielte diese Studie darauf ab, einen polyklonalen Antikörper gegen humanes Filaggrin bei atopischen Beagles, die auf Hausstaubmilben (HDM) sensibilisiert waren, und bei normalen Hunden zu

evaluieren. Die Auswirkungen einer Exposition zu Hausstaubmilben (HDM) auf Immunfärbungen und die klinische Symptomatik wurden in beiden Gruppen evaluiert. Eine positive Immunfärbung mit Anti-Filaggrin Antikörper wurde sowohl objektiv als auch subjektiv von zwei geblindeten Untersuchern beurteilt. Ein Pearson-Korrelationstest zeigte eine signifikante Korrelation zwischen objektiven und subjektiven Werten, sowohl bei den Ausgangswerten, wie auch nach der Exposition zu Allergenen ($r = 0,80$ und $P = 0,0017$ bzw. $r = 0,75$ und $P = 0,013$). Die Varianzanalyse zeigte eine signifikante Auswirkung der Zeit ($P = 0,01$), wobei die Immunfärbung bei den Ausgangsproben stärker war als nach Exposition zu HDM. Es zeigte sich auch eine signifikante Gruppe x Zeit Interaktion ($P = 0,02$), wobei sich die Immunfärbung bei atopischen Hunden mit der Zeit nicht signifikant veränderte, während sie bei normalen Hunden nach Exposition zu HDM abgenommen hatte. Ein unabhängiger t Test zeigte, dass die Ausgangsproben der atopischen Beagles signifikant weniger Immunfärbung zeigten, als die der Kontrolltiere ($P = 0,009$) und dass zwischen den Gruppen nach HDM Exposition kein signifikanter Unterschied bestand. Es bestand keine Korrelation zwischen den klinischen Bewertungen und der Immunfärbung. Bei atopischen Hunden war die Immunfärbung durch eine schwache granuläre Färbung gekennzeichnet, während normale Proben eine deutlich abgegrenzte, intensive Färbung zeigten. Darüber hinaus war die Immunfärbung in vielen Proben in allen epidermalen Schichten vorhanden, was auf eine Kreuzreaktion des benützten Antikörpers mit anderen epidermalen Proteinen, neben Filaggrin, hinweist.