

Investigation of the effect of probiotic exposure on filaggrin expression in an experimental model of canine atopic dermatitis

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Background – Atopic dermatitis (AD) results from complex interactions between an impaired skin barrier and immunological stimulation. Filaggrin is a key protein for the skin barrier, and its expression is decreased in subsets of atopic dogs and can be modified by inflammation; thus, immunomodulatory approaches may alter its expression. Probiotics have been explored for the prevention and treatment of allergies, owing to their immunomodulatory properties; however, it is currently unknown whether they can modulate filaggrin expression.

Objective – The purpose of this study was to evaluate whether probiotics can modulate filaggrin expression in an experimental model of canine AD.

Animals and methods – Eighteen atopic (11 probiotic exposed and seven control) and five normal beagles were challenged for three consecutive days with *Dermatophagoides farinae*. Skin biopsies were taken before (day 0), at the peak (day 3) and after the end of the allergen challenge (day 10). Immunohistochemistry for filaggrin was done using a polyclonal antibody specific for canine filaggrin, and staining was scored both subjectively (for intensity, granularity and continuity) and objectively, by tracing the stratum granulosum and calculating the percentage of filaggrin per unit traced area.

Results – Analysis of variance of the percentage of filaggrin in the stratum granulosum showed a significant effect of group ($P = 0.0414$, AD < normal), time ($P = 0.0066$, days 3 and 10 > day 0) and marginal group $\times$ time interaction ($P = 0.0606$). Within the atopic group, exposure to probiotics did not change filaggrin expression. No significant differences were found in the subjective scores among groups.

Conclusions and clinical importance – It is concluded that probiotic exposure early in life does not alter filaggrin expression in this AD model.

Introduction

Atopic dermatitis (AD) is the result of a complex interaction between genetic and environmental factors. A recent review highlighted the importance of skin barrier dysfunction in both human and canine AD.¹ The increase in prevalence of human AD in past decades is thought to be linked to changes in lifestyle, including a decreased exposure to beneficial bacteria that are able to modulate the immune system.^{2,3}

In order to address the issue of bacterial exposure and its immunomodulatory effect, probiotics have been the object of great interest for their potential benefits in the prevention and/or the treatment of allergies.^{4,5} The complex immunomodulatory effects of probiotics have been intensively investigated but are not completely understood.^{6,7} A shift of the T helper (Th) balance towards a Th1 cell type and modulation of the regulatory T cells are currently the most accepted mechanisms.⁸ Cytokine

modulation could also have a secondary beneficial effect on skin barrier function.

Of all the components of the skin barrier, filaggrin has been reported to be one of the most important proteins for proper keratinization.⁹ Loss-of-function mutations affecting the C-terminal portion of the filaggrin (*FLG*) gene are one of the best confirmed risk factors for the development of AD in human patients.¹⁰ Indeed, *FLG* mutations are associated with early onset and severe clinical disease.¹¹ These mutations significantly worsen skin barrier function and increase the risk for allergic sensitization.^{12,13} Filaggrin expression is not only regulated by genetics but also by inflammation.¹⁴ Cytokines that are over-represented in acute AD lesions have been reported to downregulate filaggrin expression, including interleukin (IL)-4 and IL-13,¹⁵ tumour necrosis factor- α ¹⁶ and IL-22¹⁷, with additional impact on skin barrier function. Modulation or suppression of Th2 cytokines could, therefore, be beneficial and secondarily improve skin barrier function. To date, no studies have been published on the relationship between administration of probiotics and expression of filaggrin, either in human patients or in dogs.

Preliminary studies in dogs with AD have indicated decreased filaggrin protein expression when using an

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anti-canine antibody directed at the C-terminal portion of the protein.¹⁸ Owing to the complexity of AD, experimental models are valuable for evaluating the multifaceted relationship between skin barrier impairment and the role played by allergens and immune modulation. An experimental model for canine AD has been validated in beagle dogs and includes decreased staining for filaggrin.^{19–21} The availability of the experimental model allows investigation of the effects of prenatal administration of probiotics, while controlling all other confounding factors, such as environment, diet and allergen exposure. In preliminary studies using this model, administration of *Lactobacillus rhamnosus* strain GG significantly decreased allergen-specific IgE in atopic beagles, suggesting modulation of Th2 cytokines; probiotic-exposed puppies developed less severe clinical signs.²² It is currently unknown whether probiotic administration in dogs can modulate proteins such as filaggrin.

The purpose of this study was to investigate whether exposure to probiotics early in life had an effect on filaggrin expression in this experimental model.

Materials and methods

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

Animals

Eighteen atopic beagles and five healthy beagles (normal control animals) were used; the atopic dogs had previously been epicutaneously sensitized to the house dust mite (HDM) *Dermatophagoides farinae* and were known to develop skin lesions and pruritus upon allergen exposure.¹⁹ Within the atopic group, 11 dogs had been exposed to probiotics both prenatally and postnatally for the first 6 months of life, while seven of them were not, as previously described.²² Briefly, the two atopic groups were two litters from the same parents, born 1 year apart from each other. When the bitch was pregnant with her first litter, she did not receive probiotics; the offspring from this pregnancy were considered to be the control litter.²² During the second pregnancy, the bitch received 10 capsules per day of a probiotic containing *L. rhamnosus* strain GG Culturelle® (Amerifit Brands/Culturelle; Cromwell, CT, USA), starting at week 3 of gestation and continuing throughout lactation. One capsule of the commercial product contained a minimum of 20×10^9 colony-forming units. The offspring from the second pregnancy (probiotic litter) received five capsules per day, starting at 3 weeks of age (as soon as they could eat some solid food), and this treatment continued until 6 months of age.²²

The healthy control dogs were selected to match, as closely as possible, the age and sex of the atopic beagles. These dogs had no history of skin disease, lack of evidence of skin lesions on physical examination and lack of reactivity after intradermal injection of HDM and environmental challenge with HDM (pilot studies; results not shown). The control dogs were obtained from another research colony. Both the AD and the healthy dogs were housed in the same environmental and nutritional conditions in the same research building.

Allergen challenge

Allergen challenges were performed when probiotic-exposed beagles were 1 year old and the control atopic litter (nonprobiotic exposed) was 2 years old. All beagles (healthy and AD) were environmentally challenged with HDM antigens, as previously described.¹⁹ To control for the potential effects of HDM on filaggrin expression, even in the absence of an allergic reaction, the healthy beagles were challenged in a similar manner. Briefly, HDM was applied daily for 3 days on the floor of a plastic air kennel at a dose of

50 mg/day per dog. Each dog was placed in the kennel for 3 h/day for three consecutive days. Kennels were washed at the end of the challenge. The concentration and volume of HDM used was based on previous studies.¹⁹

Skin biopsy samples

Two 8 mm punch biopsies were taken from each dog before allergen challenge (day 0), 3 days after the beginning of the challenge (day 3) and on day 10 (after the allergen challenge had been completed). All samples were taken from the inguinal or ventral thoracic areas to minimize variations due to body location; these areas were selected because they are easy to sample and, based on previous studies, these areas consistently show lesions after allergen challenge.¹⁹ The 3 day time frame was selected based on previous studies.¹⁹ Biopsies were collected after administration of 1% lidocaine for local anaesthesia and sutured routinely.

Antibody preparation

Polyclonal anti-canine filaggrin antibody was synthesized as previously described.²¹ Briefly, the canine anti-filaggrin antibody was based on the genetic sequences previously identified: (http://www.ensembl.org/Canis_familiaris/Gene/Summary?g=ENSCAFG00000023034).

Based on the genetic and amino acid sequences, the most immunogenic epitopes of the C-terminus were chosen, and a synthetic peptide was created. The amino acid sequences were then analysed against the BLAST database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) for comparative evaluation. The specificity of the anti-canine-filaggrin antibody was tested by immunoabsorption on tissue sections with complete signal inhibition and by western blotting.²¹

Immunohistochemistry: processing of samples

The 8 mm punch biopsies from the inguinal or thoracic area were fixed in 10% neutral buffered formalin for 48 h and then transferred into phosphate-buffered saline (PBS) until dehydrated and embedded in paraffin. Sections obtained on days 0, 3 and 10 were embedded in the same block for each dog. Sections were cut at 5 µm and placed on Superfrost positively charged slides (Fisher Scientific, Pittsburg, PA, USA). Slides were deparaffinized, rehydrated to PBS, then placed in Tris–EDTA buffer, pH 9.0, for 10 min in sealed tubes, which were then boiled in a water bath for 10 min, removed and set at room temperature for 10 min to unmask antigenic sites. After washing in PBS, slides were quenched in 3% hydrogen peroxide in methanol, washed in PBS, then blocked in Power block from BioGenex HRP kit (BioGenex, San Ramon, CA, USA), postblocked in normal goat serum, and then primary filaggrin polyclonal antibody, made from filaggrin canine peptide sequence, diluted at 1:1000, was added for 1.5 h. The primary antibody was detected using the BioGenex HRP kit and visualized with Nova Red (filtered). Slides were stained in two groups, with normal dogs mixed in with atopic dogs.

Immunohistochemistry: imaging and image analysis

Slides were viewed using a Nikon Lapophot microscope with ×20 objective, and 18 pictures per slide were taken for evaluation. The pictures were representative of 18 consecutive optical fields, progressing from the left to the right side of the biopsy. Images were taken with a Nikon Coolpix 990 camera and saved as JPEG files. Filaggrin expression was measured both objectively and subjectively.

Objective measurements

Semi-quantification was performed using the public domain ImageJ program (<http://rsb.info.nih.gov/ij/>) to quantify the extent of immunohistochemical staining for filaggrin in the epidermis, as previously described.^{23–26} A constant pixel-intensity threshold was set for the evaluation of every slide. The stratum granulosum was traced. The number of positive pixels and the total pixel count for the selected area were used to calculate the percentage of immunostaining in the stratum granulosum (Figure 1).

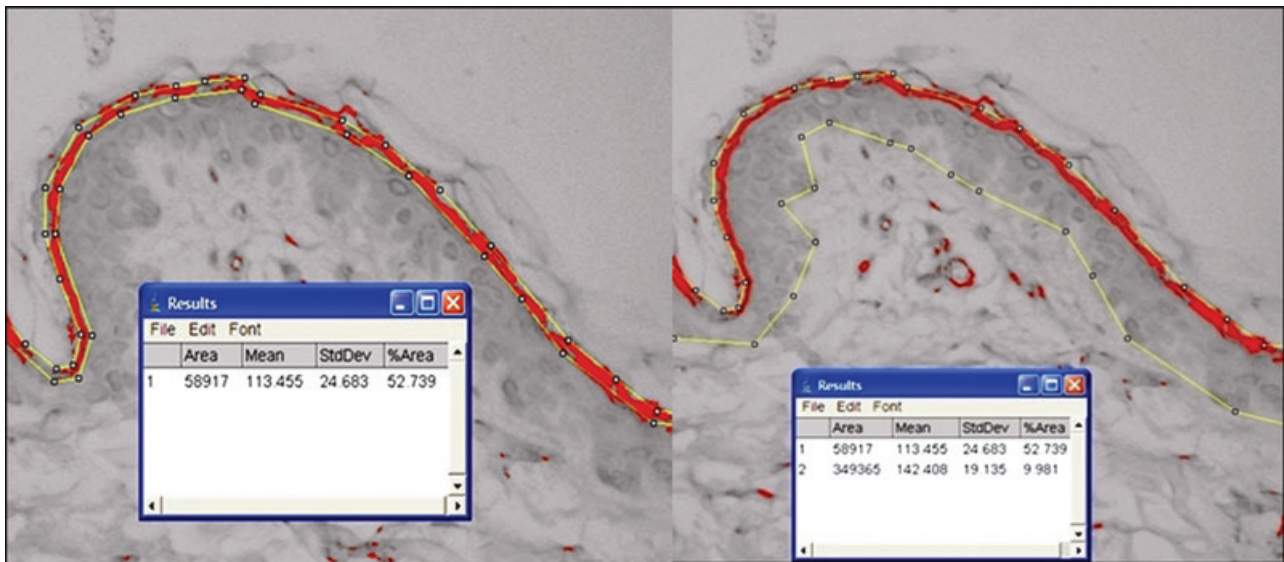


Figure 1. This figure shows the tracing of the stratum granulosum used for the objective measurements undertaken for this study. Image J was the program used for the measurements.

Subjective evaluation

Photographs were evaluated blindly by three investigators and scored for continuity, intensity and granularity on a scale of 0–3 (0 = absent, 1 = mild, 2 = moderate and 3 = severe). The score of extent and intensity of staining were added, and the mean of the total scores was calculated and used for statistical analysis.

Statistics

Spearman's rank correlation coefficient (ρ) was used to evaluate correlation between the objective and subjective scoring systems. Immunostaining scores at various time points for the different groups were analysed using a mixed-model ANOVA. Follow-up analysis for each day was performed using Student's paired *t*-tests. A *P*-value <0.05 was considered significant. For data analysis, JMP 9.0.3 (SAS Institute, Inc., Cary, NC, USA) was used.

Results

Staining was detected only in the stratum granulosum. The morphology of staining in skin samples from atopic dogs was variable, depending on the specific area examined. Figure 2 shows representative images of skin sections from normal and atopic dogs at various time points.

Objective measurements: filaggrin in stratum granulosum

For the percentage of filaggrin per unit stratum granulosum area (Figures 3 and 4), ANOVA showed a significant effect of group ($P = 0.0414$, AD < normal), a significant effect of time ($P = 0.0066$, days 3 and 10 > day 0) and a

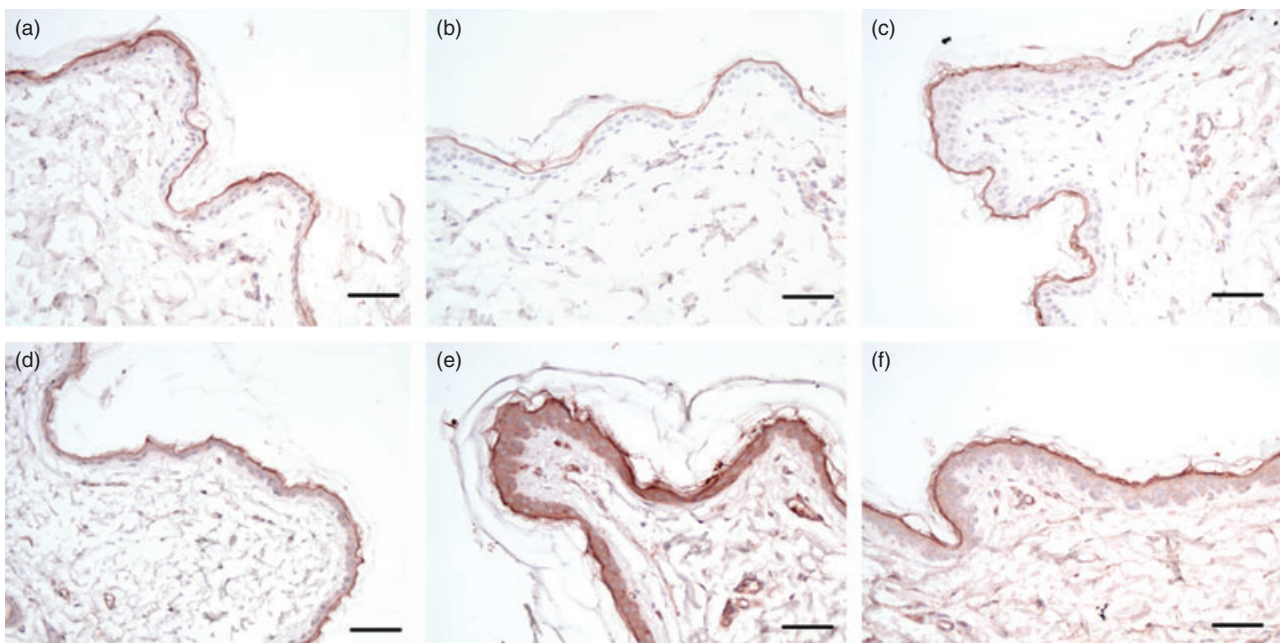


Figure 2. This composite shows pictures of skin biopsies taken from atopic dogs (top row) and normal dogs (bottom row). In atopic beagles, (a), (b) and (c) were taken on days 0, 3 and 10, respectively. In normal beagles, (d), (e) and (f) were taken on days 0, 3 and 10, respectively. Scale bar indicates 50 μ m.

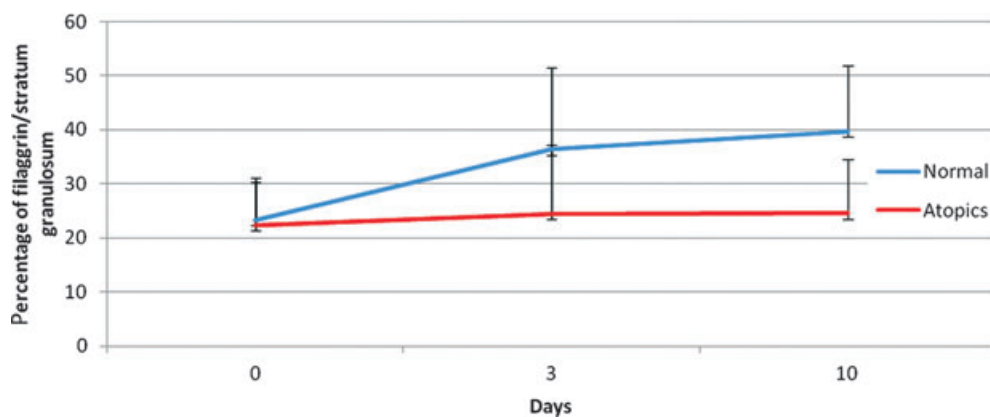


Figure 3. Figure showing the objective scores for the percentage of filaggrin per unit stratum granulosum area in normal and atopic dogs. A significant effect of group ($P = 0.0414$, AD < normal), a significant effect of time ($P = 0.0066$, days 3 and 10 > day 0) and a marginal group $\times$ time interaction ($P = 0.0606$) were found.

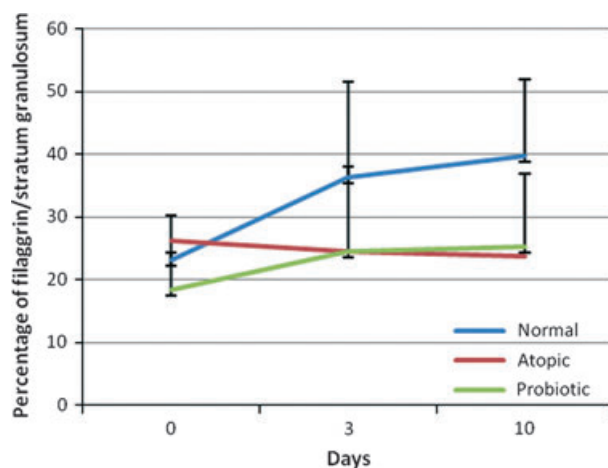


Figure 4. Figure showing the objective scores for the percentage of filaggrin per unit stratum granulosum area in normal dogs, in atopic dogs exposed to probiotics (indicated as 'probiotic') and in atopic control dogs (indicated as 'atopic'). No significant differences were found within the atopic group.

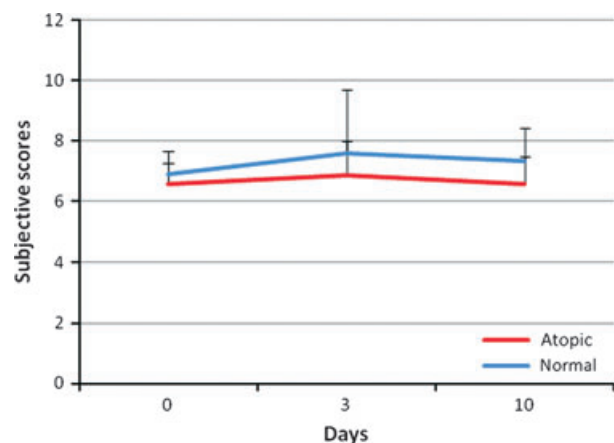


Figure 5. Figure showing the subjective scores for filaggrin immunostaining in normal and atopic dogs, where atopic dogs are not further subdivided. No significant effects of group, time, or group $\times$ time interaction were found.

marginal group $\times$ time interaction ($P = 0.0606$). Follow-up Student's paired t -tests showed no significant differences in filaggrin expression on days 0 and 3 between normal and atopic dogs, while on day 10 the values for normal dogs were significantly higher than those for atopic dogs ($P = 0.02$). No differences were found within the atopic group between probiotic-exposed atopic beagles and the atopic control dogs at any time point.

Subjective scores

No significant differences were found between groups and over time for the subjective scores (Figure 5).

Correlations between the subjective versus objective measurements

Spearman's ρ showed a significantly positive correlation between the subjective and the objective measurements of the percentage of filaggrin in the stratum granulosum ($\rho = 0.74$; $P = 0.0004$).

Discussion

In this study, experimental atopic beagle dogs had significantly decreased filaggrin immunohistochemical staining compared with normal control dogs, which is in keeping with previous reports in dogs.^{18–20} Whether decreased filaggrin staining is the result of a primary skin barrier defect or secondary to inflammation is unknown at this time.

Probiotic administration did not increase filaggrin expression in our study. While probiotic administration had shown a beneficial clinical effect in this model, such a sustained positive effect may be due to the modulation of T-regulatory cells²⁷ rather than modulation of filaggrin. The relationship between inflammation and the skin barrier is highly complex, and these two aspects are more interrelated than originally thought.²⁸ It is, however, important to point out that all *in vitro* studies on the modulation of filaggrin synthesis have involved suppression rather than stimulation and were all performed using normal human keratinocytes.^{15,16,29} Thus, while it is clear that some cytokines can suppress filaggrin expression in

normal keratinocytes, it is not yet known whether filaggrin expression can be stimulated by keratinocytes from human atopic skin lesions. One study using topical urea in normal volunteers showed increased mRNA expression of filaggrin, but no measurement of the protein was performed.³⁰ It is therefore presently unknown whether filaggrin synthesis can be increased in atopic humans. It is also unclear whether this would have a beneficial clinical effect, because the issue with filaggrin may not simply be one of quantity but also of protein quality.

The lack of effect of exposure to probiotics on filaggrin expression may be due a variety of factors. These may include an inability to suppress Th2 type cytokines sufficiently at the cutaneous level or that filaggrin expression cannot be boosted in atopic dogs, possibly because of increased proteolysis. Increased filaggrin mRNA expression in atopic dogs has been reported, compared with normal control animals, possibly due to increased proteolysis.^{31,32} Future studies using cell cultures are therefore necessary to investigate the conditions required for atopic dog keratinocytes to produce more filaggrin. Future studies should also investigate cutaneous cytokines in these two groups (probiotic exposed and control) of atopic beagles. The only study done to evaluate the effect of probiotics on cytokine profile in this model was performed on peripheral blood mononuclear cells²⁷ and not on skin biopsies. That study reported a long-lasting effect on IL-10, possibly consistent with modulation of the T-regulatory cells. The area of the complex relationship between inflammation and the skin barrier is still in its infancy in veterinary dermatology, and it is possible that other proteins in dogs are more sensitive indicators of skin barrier function besides filaggrin.

Another reason for the inability to detect differences in filaggrin expression between the atopic groups in this study may lie in the small number of dogs and the methodology used. Controlled studies of this kind, with prenatal exposure to probiotics, are expensive to carry out and, although it would have been better to have more litters and a larger number of dogs to study, financial constraints limited the number of litters. Immunohistochemistry and the use of imaging programs such as ImageJ are considered to provide a semi-quantitative approach to filaggrin quantification^{24–26} but may not be as sensitive as extraction and quantification of the protein. The authors had explored alternative options for the measurement of the protein, but studies in the literature on the extraction of filaggrin and its measurement were not found.

In conclusion, in an experimental model of canine AD early probiotic exposure did not alter filaggrin expression as determined by immunohistochemistry. Further studies are needed to elucidate the relationships between probiotic exposure and filaggrin expression.

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Résumé

Contexte – La dermatite atopique (AD) résulte d'interactions complexes entre une barrière cutanée déficiente et une stimulation immunologique. La filaggrine est une protéine clé de la barrière cutanée et son expression est diminuée chez les chiens atopiques et peut être modifiée par l'inflammation; ainsi, les approches immunomodulatrices peuvent modifier son expression. Les probiotiques ont été étudiés pour la prévention et le traitement des allergies en raison de leurs propriétés immunomodulatrices; cependant, on ignore actuellement s'ils peuvent moduler l'expression de la filaggrine.

Objectif – Le but de cette étude était d'estimer si les probiotiques peuvent modifier l'expression de la filaggrine dans un modèle expérimental de dermatite atopique canine.

Sujets et méthodes – Dix huit beagles atopiques (11 exposés aux probiotiques et sept contrôles) et cinq beagles sains ont été testés pendant trois jours consécutifs avec *Dermatophagoides farinae*. Des biopsies cutanées ont été réalisées avant (jour 0), au pic (jour 3) et après la fin du test allergénique (jour 10). L'immunohistochimie de la filaggrine a été réalisée avec des anticorps polyclonaux spécifiques de la filaggrine canine. La coloration a été évaluée subjectivement (pour l'intensité, la granularité et la continuité) et objectivement en traçant la couche granuleuse et en calculant le pourcentage de filaggrine unitaire par zone tracée.

Résultats – L'analyse de la variance du pourcentage de la filaggrine dans la couche granuleuse a montré un effet significatif du groupe ($P = 0.0414$, AD < normal), du temps ($P = 0.0066$, jours 3 et 10 > jour 0) et l'interaction marginale du groupe $\times$ temps ($P = 0.0606$). Au sein du groupe atopique, l'exposition aux probiotiques n'a pas modifié l'expression de la filaggrine. Aucune différence significative n'a été trouvée dans les notes subjectives entre les groupes.

Conclusions et importance clinique – On en conclut que l'exposition aux probiotiques tôt dans la vie n'altère pas l'expression de la filaggrine dans ce modèle d'AD.

Resumen

Introducción – La dermatitis atópica (AD) resulta tras complejas interacciones entre una función de barrera anormal en la piel y estímulos inmunológicos. La filagrina es una proteína clave para la función de barrera de la piel, y su expresión está disminuida en algunos grupos de perros atópicos y puede ser modificada por la inflamación; así pues tratamientos inmunomoduladores podrían alterar su expresión. Se ha explorado la posibilidad de utilizar probióticos para la prevención y tratamiento de alergias, dadas sus propiedades inmunomoduladoras; sin embargo se desconoce si pueden modular la expresión de filagrina.

Objetivo – El propósito de este estudio fue evaluar si los probióticos pueden modular la expresión de filagrina en un modelo experimental de AD.

Animales y métodos – Dieciocho perros de raza Beagle atópicos (11 tratados con probióticos y siete control) y cinco perros Beagle normales fueron expuestos durante tres días consecutivos a *Dermatophagoides farinae*. Se tomaron biopsias de la piel antes (día 0), en el pico (día 3) y al final de la exposición a alérgeno (día 10). Se utilizó la técnica de inmunohistoquímica para detectar la expresión de filagrina utilizando un anticuerpo policlonal específico para la filagrina canina, y la tinción de evaluó subjetivamente (para intensidad, granularidad y continuidad) y objetivamente, mediante un trazo del estrato granuloso y cálculo del porcentaje de filagrina por unidad de área trazada.

Resultados – El análisis de varianza del porcentaje de filagrina en el estrato granuloso mostró un efecto significativo en función del grupo ($P = 0,0414$, AD < normal), tiempo ($P = 0,0066$, días 3 y 10 > día 0) y una interacción marginal grupo $\times$ tiempo ($P = 0,0606$). En el grupo atópico, el tratamiento con probióticos no cambió la expresión de filagrina. No hubo diferencias significativas en la valoración subjetiva entre los grupos.

Conclusiones e importancia clínica – concluimos que el tratamiento con probióticos a edad temprana no altera la expresión de filagrina en este modelo de AD.

Zusammenfassung

Hintergrund – Die atopische Dermatitis (AD) ist das Ergebnis komplexer Interaktionen zwischen einer beeinträchtigten Hautbarriere und immunologischer Stimulation. Filaggrin ist ein Schlüsselprotein der Hautbarriere, seine Expression ist in Untergruppen von atopischen Hunden vermindert und kann durch Entzündung verändert werden; daher könnten immunmodulatorische Vorgänge seine Expression verändern. Probiotika sind aufgrund ihrer immunmodulatorischen Eigenschaften für die Prävention und zur Behandlung von Allergien untersucht worden; es ist jedoch momentan nicht bekannt, ob sie die Filaggrin Expression verändern können.

Ziel – Das Ziel dieser Studie war eine Evaluierung von Probiotika im Bezug auf ihre Fähigkeit, die Filaggrin Expression in einem experimentellen Modell der caninen AD zu modifizieren.

Tiere und Methoden – Achtzehn atopische (11 mit Verabreichung von Probiotika und sieben Kontrollen) und fünf normale Beagles wurden an drei aufeinanderfolgenden Tagen mit *Dermatophagoides farinae* stimuliert. Hautbiopsien wurden vorher (Tag 0), am Höhepunkt (Tag 3) und am Ende der Allergenstimulierung (Tag 10) entnommen. Es wurde eine immunhistochemische Untersuchung auf Filaggrin mit Hilfe eines polyklonalen Antikörpers, der spezifisch für canines Filaggrin war, durchgeführt. Die Färbung wurde sowohl subjektiv (auf Intensität, Granularität und Kontinuität) als auch objektiv beurteilt, wobei das Stratum granulosum durchgesehen wurde und der Prozentsatz von Filaggrin pro durchgesehenem Gebiet kalkuliert wurde.

Ergebnisse – Eine Varianzanalyse des Filaggrin im Stratum granulosum zeigte einen signifikanten Einfluss der Gruppe ($P=0,0414$, AD < normal), der Zeit ($P=0,0066$, Tage 3 und 10 > Tag 0) und der marginalen Gruppe x Zeit Interaktion ($P=0,0606$). Innerhalb der atopischen Gruppe änderte die Verabreichung von Probiotika die Filaggrin Expression nicht. Es wurden keine signifikanten Änderungen in der subjektiven Bewertung zwischen den Gruppen gefunden.

Schlussfolgerungen und klinische Bedeutung – Es kann hieraus geschlossen werden, dass frühzeitig im Leben verabreichte Probiotika die Filaggrin Expression in diesem AD Modell nicht verändern.

要約

背景 – アトピー性皮膚炎(AD)は皮膚のバリア傷害と免疫的な刺激の複雑な相互作用の結果によって生じる。フィラグリンは皮膚のバリアにおける重要な蛋白質で、その発現はアトピー犬の一部で減少し、炎症によっても修飾を受ける。したがって免疫調節的なアプローチがその発現を変える可能性がある。プロバイオティクスは、それらが免疫調整作用を有するために、アレルギーを予防し治療することができると探索されてきた。しかし、それらがフィラグリンの発現を調節するかどうかは未だ知られていない。

目的 – この研究の目的は実験的な犬ADモデルで、プロバイオティクスがフィラグリン発現を調節することができるかどうかを評価することである。

供与動物と方法 – 18頭のアトピー(11頭がプロバイオティクスを投与、7頭が対照)と5頭の正常ビーグルを連続した3日間 *Dermatophagoides farinae*に感作した。皮膚生検材料は感作前(0日目)、最大(3日目)とアレルゲン感作後(10日目)に採取した。フィラグリンの免疫組織化学染色はイヌフィラグリン特異的ポリクローナル抗体を用いて行い、染色を主観的(濃さ、粒度と連続性)ならびに客観的に顆粒層の面積当たりのフィラグリンの割合を計算することでスコア化した。

結果 – 顆粒層におけるフィラグリンの割合の分散の解析は群($P = 0.0414$, AD < 正常)、時間($P = 0.0066$, 3日目と10日目 > 0日目)、限界の群×時間相互作用($P = 0.0606$)の有意な効果を示していた。アトピー群内では、プロバイオティクスへの暴露はフィラグリンの発現を変化させなかった。客観的なスコアは群間で有意差はみられなかった。

結論と臨床的な重要性 – 若年期でのプロバイオティクスの暴露はADモデルにおいてフィラグリンの発現を変化させないという結論であった。

摘要

背景 – 异位性皮膚炎(AD) 起因于受損的皮肤屏障和免疫刺激间复杂的相互作用。丝聚蛋白是皮肤屏障中一种关键蛋白质,其表达可使异位性疾病患犬亚型减少,并可因炎症变化;因此,免疫调节方法可能改变其表达。益生菌由于其免疫调节性能,被开发用于防治过敏症;然而,目前未知它是否能够调节丝聚蛋白表达。

目的 – 本研究目的是评估益生菌是否能调节犬异位性皮肤炎试验模型丝聚蛋白的表达。

动物和方法 – 18只异位性(11只益生菌,7只对照)和5只正常比格犬接受连续3日的粉尘螨试验。在实验前(0日)、峰值(3日)和过敏原激发最后(10日)进行皮肤活检。丝聚蛋白免疫组化使用一个特定的犬丝聚蛋白多克隆抗体,染色进行主观(强度、颗粒度和连续性)和客观评分,追踪颗粒层并计算追踪地区每单位丝聚蛋白百分比。

结果 – 颗粒层丝聚蛋白百分比方差分析显示效果显著,组间($P = 0.0414$, AD < 正常),时间($P = 0.0066$, 3日和10日 > 0日),边缘组×时间相互作用($P = 0.0606$)。在异位性疾病组中,使用益生菌不能改变丝聚蛋白表达。组间主观评分未发现显著差异。

结论和临床价值 – 结论是在异位性疾病模型中益生菌使用早期不改变丝聚蛋白表达。