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Mini review

Is the skin barrier abnormal in dogs with atopic dermatitis?

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ABSTRACT

In mammalian skin, the stratum corneum exerts a barrier function that protects from transepidermal water loss and the penetration of exogenous molecules, such as allergens, from the environment. Recently, skin barrier defects have been shown to be of prime importance in the pathogenesis of human atopic dermatitis. In this review, we summarize the latest research data pertinent to the stratum corneum and barrier function of dogs with atopic dermatitis. At the time of this writing, there is increasing evidence that a skin barrier defect likely exists in dogs with atopic dermatitis. This barrier dysfunction is characterized by abnormal intercellular stratum corneum lipid lamellae, abnormal stratum corneum morphology, reduced and abnormal ceramide content and, in some but not all dogs, abnormal filaggrin expression. In association with these changes, there is higher transepidermal water loss in atopic than in normal canine skin. Furthermore, atopic inflammation appears to worsen transepidermal water loss and filaggrin expression. It remains unknown, however, if the changes observed are primary (i.e. of genetic origin) or secondary to atopic inflammation that also exists even in clinically normal skin. Finally, whether or not a therapeutic intervention aimed at restoring a dysfunctional skin barrier is of any clinical benefit to atopic dogs has not yet been proven unequivocally.

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1. Introduction

An intact skin barrier is critical to prevent the desiccation from excessive water loss and the penetration of exogenous substances detrimental to the body. The main responsibility for assuming this protective role lies with the outermost epidermal layer, the stratum corneum (SC), a zone composed of cornified keratinocytes (corneocytes) surrounded by complex lipid lamellae (Madison, 2003; Elias and Schmuth, 2009). In recent years, the SC and its barrier function have taken center stage in the pathogenesis of atopic dermatitis (AD) in humans (Elias et al., 2008; Cork

et al., 2009; Elias and Schmuth, 2009). The most important link between barrier dysfunction and AD came after the unexpected discovery that mutations in the filaggrin gene *FLG* were not only causative of human ichthyosis vulgaris, but also predisposing to AD in some patients (Palmer et al., 2006; Smith et al., 2006). A recent systematic review and meta-analysis established that *FLG* gene mutations increased the risk of developing allergic sensitizations, AD and allergic rhinitis or asthma associated with AD (van den Oord and Sheikh, 2009). Not only do some humans with AD have *FLG* loss-of-function mutations, but there is evidence that, by itself, type-2 pro-allergic cytokine secretion in the skin of affected patients reduces the expression of filaggrin and other cornified envelope proteins such as involucrin and loricrin (Howell et al., 2007, 2009; Kim et al., 2008). As a result of these observations, the new “outside-inside-outside” theory for the pathogenesis of human AD postulates that a genetic skin barrier defect leads to atopic cutaneous inflammation that further results in decreased

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SC protein secretion and aggravated barrier dysfunction (Elias and Schmuth, 2009).

Ten years ago, we published in this journal a short review on skin lipids as part of the special issue reporting the work of the American College of Veterinary Dermatology Task Force on Canine AD (Olivry and Hill, 2001). At that time, only six studies evaluating this subject were briefly reviewed, and there was no information available on non-lipid skin components. Because of the latest change in paradigm and focus regarding the pathogenesis of human AD, the question whether or not a cutaneous barrier dysfunction exists in dogs with the homologous disease is relevant and timely. This mini-review will critically examine the evidence existing for skin barrier anomalies in dogs with AD. We will successively discuss the changes in skin permeability, SC lipids, superficial epidermal proteins, the implications of skin barrier dysfunction on the process of sensitization and the results of trials employing topical formulations aimed at skin barrier restoration.

2. Do dogs with atopic dermatitis have dry skin and lose water through their skin?

In humans, the assessment of skin barrier function has traditionally relied on the measurement of transepidermal water loss (TEWL) with either open or closed chamber evaporimeters. There is indisputable evidence that patients with AD have increased TEWL in normal-appearing skin and that skin lesions and higher disease severity increase these values (Linde, 1992; Gupta et al., 2008). At this time, the usefulness of TEWL measurements in dogs remains debatable, either with open or closed chamber devices, as there is very high day-to-day, site-to-site and dog-to-dog variability that makes comparisons between patients unreliable in clinical studies (Beco and Fontaine, 2000; Lau-Gillard et al., 2010).

In spite of the limitations stated above, Shimada and colleagues reported that, in ten dogs with spontaneously arising AD, closed-chamber TEWL measurements of both clinically nonlesional and lesional skin were markedly higher than those of normal dogs at similar sites (Shimada et al., 2009). In contrast, skin moisture, which was assessed by electrical capacitance, was comparable between normal dog and nonlesional AD skin while it was lower in lesional AD skin (Shimada et al., 2009).

Similarly, Hightower and Marsella reported that atopic beagles experimentally sensitized to house dust mites (HDM; $n=24$) had significantly higher open-chamber TEWL values at six of ten clinically normal body sites compared to normal beagles ($n=21$) (Hightower et al., 2010). Interestingly, five of these six sites are normally prone to develop AD skin lesions. Furthermore, challenges with HDM resulted in significantly higher TEWL values in young dogs, and these measurements were highest at AD predilection sites that normally develop mite-induced skin lesions (Hightower et al., 2010).

In summary, measuring TEWL in dogs does not appear to be the most optimal method to assess skin barrier function in dogs due to the very high variability and lack of reproducibility of measurements. In spite of this caveat, values from dogs with spontaneous AD seem markedly

higher than those of normal dogs, with measurements in lesional skin exceeding those of visibly normal skin. In an experimental dog model of AD skin lesion development, allergenic challenge appears to increase TEWL values compared to pre-provocation ones, and this is most prominent at AD predilection sites. In contrast, skin hydration appears comparable between normal canine skin and nonlesional AD skin. Lesional atopic skin exhibits low moisture values.

3. Do dogs with atopic dermatitis have abnormal stratum corneum lipids?

3.1. Anomalies of stratum corneum lipid lamellae

A decade ago, we reported the morphology of intercellular lipid lamellae in the clinically nonlesional SC of five dogs with AD and compared it to that of five normal dogs (Inman et al., 2001). Ruthenium tetroxide fixation permitted the visualization of intercellular lipids as multilayered lamellae at all levels of normal SC. The deposition of lipid lamellae in the SC of dogs with AD was very heterogeneous compared with that of normal canine skin. Many areas of atopic SC were found to be devoid of lipid lamellae. When present, the lamellae often exhibited an abnormal and/or an incomplete structure. Lamellar bodies extruding lipids appeared fairly normal in number and morphology. When compared between normal and atopic canine SC, scores for continuity and thickness of intercellular lipid lamellae were found to be significantly lower in nonlesional atopic than normal canine SC (Inman et al., 2001).

Similar changes were reported recently in nonlesional SC of five dogs with naturally occurring AD (Piekutowska et al., 2008). In these dogs, intercellular spaces often were found to be devoid of lipid lamellae, and there was premature separation of corneocytes leading in wider intercellular spaces in atopic compared to normal dogs (Piekutowska et al., 2008).

Finally, in an experimental model of canine AD, wide intercellular spaces, disorganized lipid lamellae and intra-corneocyte retention of lamellar bodies were found in visibly nonlesional SC of HDM-sensitized but not normal beagles (Marsella et al., 2010). In these hypersensitive dogs, HDM allergenic challenge induced macroscopic skin lesions associated with further disorganization of corneocytes and lipid lamellae arrangement as well as increased widening of intracellular spaces in the SC (Marsella et al., 2010).

In summary, anomalies in the amount and arrangement of intercellular lipid lamellae exist in the clinically nonlesional SC of dogs with AD. Furthermore, in experimentally sensitized dogs, allergen challenge seems to further worsen SC lipid anomalies.

3.2. Anomalies of stratum corneum lipid composition

Four recent studies examined the SC lipid anomalies in dogs with AD.

In the first study, lipids were extracted from the SC of ten dogs with AD and 30 normal dogs, and they were separated by high performance thin layer chromatography

(Shimada et al., 2009). Compared to that of normal dogs, both nonlesional and lesional SC of dogs with AD had a lower proportion of ceramides but similar relative amounts of cholesterol and free fatty acids (Shimada et al., 2009). In these atopic dogs, the relative amount of SC ceramides was negatively correlated with TEWL values indicating the possible contribution of these ceramides to epidermal water permeability (Shimada et al., 2009). An important caveat of this study is that the average age of dogs with AD was more than twice that of normal dogs, and it is possible that the decrease in proportion of ceramides might not only be due to their atopic status but also to their older age.

Using a similar methodology, lipids were examined in the nonlesional SC of 14 dogs with AD and a similar number of age, breed and sex matched dogs (Reiter et al., 2009). In dogs with AD, the proportion of SC ceramides CER[EOS] (ceramide 1) and CER[EOP] (ceramide 9) was lower, and that of cholesterol higher than that of their matched normal controls (Reiter et al., 2009). Interestingly, both CER[EOS] and CER[EOP] are important carriers of linoleic acid, an omega-6 fatty acid, and these two ceramides are believed to be critical to normal SC barrier function (Mizutani et al., 2009).

Importantly, both studies above reported changes in relative percentages of ceramides among total lipid content, but changes in the quantity of ceramide themselves was not determined as the total amount of SC lipids was not measured.

Further evidence of a ceramide deficiency in atopic dogs came from the recent demonstration of a significant reduction in the quantity of SC total free ceramides and their subclasses CER[EOS], CER[NS]/[NDS] (ceramide-2/10), CER[NP] (ceramide-3), CER[AS]/[NH] (ceramides 5/8) and CER[EOP] in both lesional and nonlesional skin of ten dogs with AD compared to that of ten healthy age- and breed-matched dogs. There was no difference in the amount of ceramides between nonlesional and lesional canine AD skin. The highest decrease in ceramide content in atopic dogs was in the amount of the mixtures of CER[NS]/[NDS] and CER[AS]/[NH] (Yoon et al., 2011).

Comparing the lipid content and composition of SC samples obtained from five normal dogs and five dogs with AD, Popa and colleagues recently confirmed and expanded upon earlier observations (Popa et al., 2011). The total lipid amount was lower in the SC of atopic dogs compared to that of normal dogs. There were lower quantities of free and protein-bound ceramides with CER[EOP] and CER[AH] (ceramide-7) being barely detectable. Interestingly, there was a concurrent increase in the amounts of both free and protein-bound glycosylceramides in the SC of atopic dogs compared to normal dogs. This increase suggests the possible existence of an abnormal ceramide metabolic pathway in the skin of atopic dogs, for example a deficiency in beta-glucocerebrosidase, the enzyme converting glycosylceramides into ceramides (Popa et al., 2011). Finally, the quantity of SC protein-bound omega-hydroxyceramides was very low in atopic dogs with CER[OS] being the only ceramide detected. This low amount of bound nonesterified omega-hydroxyceramide CER[OS] and CER[OP] matched the reduction in their

esterified precursors CER[EOS] and CER[EOP]. Of importance is the observation of a noticeable variation in the lipid composition between SC layers in dogs with AD, suggesting that successive waves of inflammation might transiently have altered lipid biosynthesis (Popa et al., 2011).

Finally, Bäumer et al. reported recently that the lesional skin of five dogs with AD had markedly lower amounts of the natural anti-inflammatory ceramide-degradation product sphingosine-1-phosphate (S1P) (Bäumer et al., 2011). Furthermore, exogenously added S1P was removed more rapidly from atopic than normal skin biopsies, thereby indicating a possible higher activity of S1P lyase 1, the S1P-degrading enzyme (Bäumer et al., 2011). In parallel to the observations made above from skin biopsies, plasma or serum levels of S1P were also significantly lower in atopic compared to normal dogs (Bäumer et al., 2011). Increases in the transcription rates of S1P-lyase-encoding *SGPL1* have already been reported in both nonlesional and lesional canine AD compared to normal canine skin, and this higher expression is a logical explanation for the reduction in S1P levels and its higher degradation rates seen in this disease (Wood et al., 2009).

In summary, there is a reduction in total ceramides and some free and protein-bound ceramide subclasses in the nonlesional SC of dogs with AD. This ceramide reduction is associated with increased TEWL. Finally, there is a decrease skin and plasma amount of the anti-inflammatory ceramide degradation product S1P, and this could be due to higher transcription rate of its degrading enzyme, the S1P lyase 1. The pathogenic relevance of these observations is unknown.

4. Do dogs with atopic dermatitis have abnormal stratum corneum proteins?

Three studies have investigated the expression of filaggrin in the skin of dogs with AD and in experimentally induced canine atopic skin lesions.

In 2009, lesional skin biopsies from beagles with experimental AD were immunostained with a polyclonal antiserum directed against human filaggrin. The observed panepidermal staining suggested that this antiserum not only recognized filaggrin itself but also other proteins such as keratins, which limits the validity of the staining results (Marsella et al., 2009). In spite of this important limitation, the authors reported a weaker immunostaining in the skin of experimental atopic lesions compared to normal canine skin (Marsella et al., 2009). It is hypothesized that this change could be due to a variation in keratin pairs expressed in an epidermis reacting to inflammation.

In 2010, Chervet and colleagues reported results of indirect immunofluorescence performed in normal skin of 16 healthy dogs and nonlesional skin of 18 dogs with AD using antibodies specific for either the carboxy or aminoterminal of filaggrin (Chervet et al., 2010). Four distinctive filaggrin expression patterns were identified in non-lesional skin. Ten dogs exhibited an identical staining pattern for both antibodies with comparable (category I; 3/18, 17%) or reduced (category II; 7/18, 39%) fluorescence intensity compared to that of controls. In contrast, 4/18 dogs (22%) displayed aberrant large vesicles revealed by the

C-terminal but not the N-terminal antibody (category III). Finally, 4/18 dogs (22%) had normal N-terminal but no detectable C-terminal filaggrin (category IV). The observation of reduced filaggrin expression in dogs from category II is compatible with a possible decrease secondary to atopic inflammation, while the anomalies seen in dogs from category IV, and possibly in those from category III, are suggestive of mutations in the *FLG* gene that result in the abnormal or loss of function of the filaggrin protein (Chervet et al., 2010).

Finally, Santoro and colleagues recently studied the expression of filaggrin in healthy and mite-sensitized beagles before and after HDM allergen challenge (Santoro et al., 2010). In both groups of dogs, filaggrin mRNA decreased in skin biopsies after allergen provocation then it increased slightly after lesions abated. After challenge, protein immunostaining appeared normal in the upper epidermis of normal dogs while it became more irregular and patchier in hypersensitive dogs. In the latter, filaggrin immunoreactivity increased after resolution of mite-induced clinical signs compared to pre-provocation values. These observations suggest that, in itself, allergen-induced atopic inflammation can reduce filaggrin expression in this experimental model of canine AD and that resolution of signs results in some recovery of these changes (Santoro et al., 2010).

At this time, linkage analyses have excluded the *FLG* gene as associated with AD in west highland white terriers in Australia (Barros Roque et al., 2009), the UK (Wood et al., 2010) and the USA (Salzmann et al., 2011). In contrast, the filaggrin gene region appears to be associated with AD in Labradors in the UK (Wood et al., 2010).

In summary, there is evidence that a fraction of dogs with AD have anomalies in their filaggrin carboxy-terminus that could be due to *FLG* mutations, and that atopic inflammation also induces transient decreases of filaggrin expression. At this time, however, mutations in *FLG* have not been confirmed to exist in dogs with this disease.

5. What are the consequences for dogs of having a defective skin barrier?

Two studies have reported the changes that occur after SC tape removal in normal and atopic canine skin.

In 2009, using a closed-chamber evaporimeter, Shimada and colleagues established that TEWL values increased linearly with increasing numbers of corneocyte-removing tape strips (Shimada et al., 2008).

Furthermore, sensitization to epicutaneously applied HDM occurred earlier and was stronger, as shown by allergen-specific peripheral blood T-lymphocyte activation, allergen-specific IgE serum levels and intradermal testing, in atopic laboratory dogs in whom the SC was removed by tape-stripping compared to dogs in whom the SC was not altered (Olivry et al., 2011).

In summary, in dogs as in other species, an intact SC appears to be a barrier indispensable for the limitation of water evaporation and cutaneous allergen sensitization.

6. Are topical lipid interventions of clinical benefit for dogs with atopic dermatitis?

At the time of this writing, there are only two reports of clinical trials testing topical lipid-based formulations aimed at restoring the skin barrier in dogs with AD.

The first blinded randomized controlled trial, reported only in abstract form, enrolled 47 dogs with AD, which were allocated to receive either a phytosphingosine-containing shampoo (Douxocalm, Sogeval), the phytosphingosine-containing shampoo then a spray with similar ingredients (Douxocalm microemulsion spray, Sogeval) or a control shampoo containing antiseptics, fatty acids and complex sugars (Allermyl, Virbac) (Bourdeau et al., 2007). Dogs were treated for 19 days. After 20 days, the CADESI lesional score had decreased in the three treatment groups by 40–46% while the pruritus grade had been reduced by 23–30%. There were no apparent differences in efficacy between these three interventions, which all had lipid ingredients theoretically capable of affecting skin barrier function. Whether or not phytosphingosine-containing topical products would offer additional treatment benefit compared to mere bathing with a non-lipid containing shampoo, with or without another emollient, was not determined due to the absence of a negative (placebo) control group.

Recently, a small randomized open trial tested the efficacy of two topical products containing essential plant oils and unsaturated fatty acids in six normal dogs and 14 dogs with AD (Tretter and Mueller, 2011). Dogs were treated either with a weekly spot-on (Dermoscent Essential 6, LDCA) or a once daily spray containing similar albeit different ingredients (Dermoscent Atop 7, LDCA). After eight weeks, the CADESI lesional scores and the pruritus visual analog values had decreased in 13/14 and 9/14 dogs with AD, respectively. Nevertheless, there were no differences in perceived effect between groups. Additionally, TEWL was measured at three different body sites, before and after treatment, using a non-validated closed-chamber evaporimeter. In all, the tested topical medications did not appear to have any effect in improving TEWL values.

In summary, the results of this very small clinical trial (less than ten atopic dogs tested with each intervention) were interpreted as in support of a possible benefit of the tested products. However, at study onset, it appears that some atopic dogs entered in this study had CADESI scores in the range of patients with AD in clinical remission (CADESI-03 lower than 16) or with very mild to mild disease (scores of 16–59) (Olivry et al., 2008). Similarly, at the beginning of this trial, some atopic dogs had pruritus values that were either in the range of those of normal dogs (1.9 or less) or that were very low (Rybnicek et al., 2009). Finally, the TEWL values reported in these atopic dogs appear to be mostly in the range of those of normal canine skin and lower than those previously reported for nonlesional and lesional AD skin (Shimada et al., 2009). Such low disease severity and fairly normal TEWL values at study onset are bound to render the interpretation of treatment effect difficult. Furthermore, the lack of a control group treated with a non-lipid-based topical formulation is another source of possible bias, as a placebo effect is commonly seen in

Table 1

Skin barrier changes in canine AD skin—2011 update.

	Nonlesional AD skin ^a	Lesional AD skin ^b	References
Skin moisture	Similar to normal skin	Lower than in nonlesional AD skin	Shimada et al. (2009)
TEWL	Values in lesional AD skin > nonlesional AD skin > normal canine skin allergen challenges increase values		Shimada et al. (2009) and Hightower et al. (2010)
Ultrastructure	Disorganized corneocytes with intercellular spaces; thinner, less common and disorganized lipid lamellae	Higher disorganization of corneocytes and lipid lamellae with lamellar bodies within corneocytes after allergen challenges	Inman et al. (2001), Piekutowska et al. (2008) and Marsella et al. (2010)
SC lipids	Lower quantity and proportion of free and protein-bound SC ceramides Lower quantity and proportion of several ceramide subclasses Higher quantity of free and protein-bound glucosylceramides Lower quantity and higher degradation of sphingosine-1-phosphate		Shimada et al. (2009), Reiter et al. (2009), Yoon et al. (2011), Popa et al. (2011) and Bäumer et al. (2011)
Filaggrin	Absent or aberrant expression of C-terminal filaggrin in ~half of dogs with AD	Allergen-challenges reduce filaggrin transcription and result in patchy staining	Chervet et al. (2010) and Santoro et al. (2010)

Abbreviations: AD: atopic dermatitis; SC: stratum corneum; TEWL: transepidermal water loss.

Unless otherwise reported, changes refer to changes compared to normal canine skin.

^a Nonlesional skin correspond to clinically normal appearing skin in dogs with AD.^b Lesional skin correspond to spontaneous lesion-bearing or post-challenge experimental AD skin.

clinical trials enrolling dogs with AD (Olivry and Mueller, 2003; Olivry et al., 2010).

In summary, two small clinical trials provided limited evidence of inconclusive benefit of lipid-based topical therapy in dogs with AD. Larger clinical trials of higher design grade (e.g. randomized blinded trials controlled with inactive placebo) should be performed to better determine the value of these and similar interventions in the treatment of canine AD.

7. Conclusions and implications for research and practice

At the time of this writing, there is increasing evidence that a skin barrier defect likely exists in dogs with AD. This barrier dysfunction is characterized by abnormal intercellular SC lipid lamellae, abnormal SC morphology, reduced and abnormal ceramide content and, in some dogs, abnormal filaggrin expression (Table 1). As a logical consequence of these barrier changes, TEWL is normally increased. Allergen-induced atopic inflammation appears to further worsen these anomalies. Finally SC removal facilitates sensitization to epicutaneously applied allergens.

It is currently not known if only some or all dogs with AD have similar skin barrier dysfunction. Furthermore, whether this defect, if present, is primary (i.e. of genetic origin) and/or secondary to atopic skin inflammation itself remains to be determined. Indeed, nonlesional (i.e. clinically normal) atopic canine skin is NOT identical to normal dog skin, as it exhibits microscopic inflammation and contains proinflammatory cytokines similar in nature to those of lesional atopic skin (Olivry et al., 1997, 1999; Nuttall et al., 2002a,b).

Future research projects should focus on assessing if the changes in lipids and proteins vary with flares of AD in clinically normal skin of dogs with spontaneous AD, and whether or not anti-inflammatory treatments can lead to a return of these changes to values in the range of those of normal dogs.

Clinically, the optimal intervention to correct a possible barrier dysfunction (e.g. nutritional lipid supplementation and/or topical lipid application) and the therapeutic benefit – if any – of correcting such barrier defect remain largely unknown and unproven. These issues must be addressed in high quality randomized controlled trials to irrefutably demonstrate the clinical benefit of products marketed for the correction of primary and/or secondary barrier dysfunction in atopic dogs. Indeed, practitioners are in need of evidence-based information on how to best select and clinically use such products for, ultimately, increasing their likelihood of success to prevent the development of, or treat canine AD skin lesions.

Conflict of interest

The author does not declare any conflict of interest relevant to this review except for research funding and consulting honorarium received from Virbac France and lecturing and consulting honorarium given by Sogeval; both companies currently market topical lipid formulations aimed at restoring skin barrier function in dogs.

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