

Review: Pathogenesis of canine atopic dermatitis: skin barrier and host–micro-organism interaction

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Background – Canine atopic dermatitis (AD) is a common, genetically predisposed, inflammatory and pruritic skin disease. The pathogenesis of canine AD is incompletely understood.

Objectives – The aim of this review is to provide an in-depth update on the involvement of skin barrier and host–microbiome interaction in the pathogenesis of canine AD.

Methods – Online citation databases and abstracts from international meetings were searched for publications related to skin barrier and host–microbiome interaction (e.g. bacteria, yeast, antimicrobial peptides).

Results – A total of 126 publications were identified. This review article focuses on epidermal barrier dysfunction and the interaction between cutaneous microbes (bacteria and yeasts) and the host (antimicrobial peptides). Epidemiological updates on the presence of pathogenic organisms and canine AD are also provided.

Conclusions and clinical importance – Major advances have been made in the investigation of skin barrier dysfunction in canine AD, although many questions still remain. Skin barrier dysfunction and host–microbiome interactions are emerging as primary alterations in canine AD. Based on this review, it is clear that future studies focused on the development of drugs able to restore the skin barrier and increase the natural defences against pathogenic organisms are needed.

Introduction

Atopic dermatitis (AD) has a multifactorial, incompletely understood pathogenesis in both dogs and people. Until a few years ago, the traditional pathogenetic view of AD focused on the interpretation that AD was due to genetic alterations of the immune system leading to abnormal immunological response [e.g. increased allergen-specific immunoglobulin E (IgE)] to innocuous allergens ('inside-outside' theory). More recently, a theory focused on the fundamental role of the epidermal barrier has been proposed ('outside-inside' theory).¹ The

'outside-inside' theory proposed that an abnormal skin barrier in atopic patients facilitates the penetration of allergens (and microbes) through the epidermis, increasing their contact and exposure to epidermal immune cells. Such theories are not mutually exclusive and they have been integrated into a more comprehensive theory (i.e. 'outside-inside-outside' theory) based on the view that a primary defect in the epidermal barrier leads to a higher penetration of allergens and microbes that overstimulate the local immunity (innate and adaptive).² Such excessive stimulation triggers the release of inflammatory mediators that further exacerbate the barrier dysfunction. In dogs, few studies have analysed the skin barrier defects and the relationship between local immunity and the cutaneous microbiome in AD. This article reviews the research on skin barrier defects and host–microbe interaction in canine AD reported in the last 12 years.

Skin barrier dysfunction

Historical perspective

In 2001, the American college of Veterinary Dermatology (ACVD) Task Force on Canine Atopic Dermatitis published a series of papers summarizing the 'state-of-the-art' knowledge about the clinical features and pathogenesis

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of atopic dermatitis. At the time, it was known that humans with AD frequently exhibited xerosis and an apparent impairment of their epidermal barrier function as demonstrated by increased transepidermal water loss (TEWL).³ This impaired function was associated with abnormalities in epidermal lipid metabolism and lamellar body extrusion, resulting in decreased ceramide levels in both lesional and nonlesional skin.^{4,5} However, relatively few studies had been performed to investigate epidermal barrier function in dogs. One early study failed to demonstrate differences in epidermal water dynamics in dogs with AD compared with healthy dogs.⁶ Nonetheless, studies investigating the ultrastructure of canine epidermis found a decreased thickness and length of the stratum corneum lipid deposits in nonlesional skin of dogs with AD.⁷ Furthermore, electron microscopy of nonlesional skin from dogs with AD revealed disrupted lipid lamellae, although impaired extrusion of lamellar bodies could not be demonstrated.⁷ These findings suggested the possibility of a structural defect in the epidermal barrier in atopic dogs, regardless of whether or not a functional defect could be demonstrated.

Similar conflicting results were obtained in studies investigating the plasma and cutaneous lipid profile in dogs with AD. In one study, decreased serum triglyceride levels were found in atopic dogs after corn oil administration, suggesting a primary defect in lipid absorption or metabolism.⁸ Likewise, a second study found that canine (and human) patients with AD had slightly higher plasma levels of linoleic acid and lower levels of dihomo- γ -linolenic acid and arachidonic acid than normal patients.⁹ It was hypothesized that these changes might be due to a decrease in Δ^6 desaturase activity. In contrast, these findings were not replicated in a study that demonstrated significantly greater levels of serum dihomo- γ -linolenic acid in dogs with AD in comparison to healthy dogs.¹⁰ Furthermore, one report investigating the serum and cutaneous lipid profiles of atopic, seborrhoeic and normal dogs failed to demonstrate any significant differences between any of the groups.¹¹ Interpretation of these contradictory results was further complicated by the finding that serum and skin lipids were significantly influenced by the fatty acid source of the diets that the dogs were eating.¹² The clinical relevance of dietary fatty acid composition to canine epidermal barrier function was further supported by work demonstrating significant decreases in TEWL in dogs supplemented with linoleic acid.¹³

Update on skin barrier dysfunction

Skin barrier dysfunction: cause or effect?

Skin barrier dysfunction is currently of great interest in both human and veterinary dermatology. The acknowledgement of the role of the skin barrier in veterinary dermatology is relatively recent, and it is unclear whether the reported skin barrier abnormalities in canine AD are primary or secondary to inflammation.^{14,15} What is known is that the skin barrier is altered in dogs with AD and that there are striking similarities between the canine and the human disease in terms of skin barrier impairment.¹⁶ This may be important in the pathogenesis of canine AD, because it increases the risk for allergic sensitization.^{17,18}

This has been demonstrated in an experimental model of canine AD, and it may play a role in the naturally occurring disease as well.¹⁷

Transepidermal water loss

Skin barrier function can be evaluated in a variety of ways. The most commonly accepted noninvasive approach is the measurement of TEWL, which increases when the skin is damaged. In veterinary medicine, measurement of TEWL has many limitations and has been reported to have questionable repeatability.¹⁹ Nevertheless, it has been demonstrated that removal of the stratum corneum leads to increased TEWL in dogs and that the increase is directly proportional to the severity of damage to the stratum corneum.²⁰

In pilot studies using an experimental model of canine AD, TEWL was increased in atopic beagle dogs compared with age-matched healthy beagles. This was particularly evident in sites prone to AD even when no clinical lesions were evident and was more marked in young individuals than in older dogs.²¹ Increased TEWL has also been reported in lesional and nonlesional skin of naturally occurring canine AD.²² Nevertheless, several studies failed to show a significant correlation between TEWL and the severity of clinical lesions (as assessed by the total Canine Atopic Dermatitis Extent and Severity Index score).²³ However, this may in part be due to limitations of TEWL measurements and does not decrease the importance of skin barrier dysfunction in canine AD.

Ceramides and lipids

Ceramides are waxy lipid molecules composed of one sphingosine and one fatty acid. These molecules are mainly located in the cellular lipid bilayer. Ceramides may be composed of combinations of different sphingosines and fatty acids, and they are named accordingly.²⁴ Ceramides function as structural elements but are also involved in cellular signalling.²⁵ Increased TEWL in dogs with AD has been associated with decreased ceramide levels.²² Ceramide deficiency is also seen in human patients with AD, where it is considered both a primary defect and a consequence of inflammation. A recent study in an experimental model of canine AD showed that the decrease of ceramides is, at least in part, linked to the acute inflammatory response triggered by allergen exposure in sensitized individuals.⁴ Interestingly, the authors reported that the selective reduction of stratum corneum ceramides was detectable at both sites of inflammation and sites away from the allergen application. Another study found that the proportion of the epidermal lipid layer consisting of ceramides 1/CER[EOS] and 9/CER[EOP] was significantly lower in nonlesional skin of dogs with AD compared with healthy dogs, while the proportion of cholesterol was significantly higher in the atopic dogs.^{22,26} These results may be interpreted as consistent with a primary ceramide abnormality in dogs with AD, although it is important to remember that clinically nonlesional skin may still exhibit low-grade inflammation. Studies on the role of genetic mutations in enzymes responsible for ceramide synthesis and degradation are therefore needed to address the issue of primary lipid defects in canine AD.

Healthy dogs of different breeds show wide quantitative variations in the patterns of stratum corneum ceramides.²⁷ Large amounts of glucosylceramides have been found among the free lipids in the stratum corneum of dogs with AD, although they are nearly absent from the stratum corneum of healthy dogs.²⁸ In the same study, the authors reported a peculiar heterogeneity of the distribution of protein-bound lipids in the stratum corneum of atopic dogs.²⁸ In particular, the local ceramide and protein-bound lipids varied in 'waves' from layer to layer, suggesting that their levels may have been altered during periods of cutaneous inflammation.²⁸ One study accounted for this by comparing the fractions of free stratum corneum ceramides in dogs with AD with breed- and age-matched healthy dogs using high-performance thin-layer chromatography.²⁹ The authors reported that ceramide 1/CER[EOS], ceramides 9/CER[EOP] and ceramide [NP], which are known to be decreased in the skin of humans with AD, were also decreased in the skin of dogs with AD.²⁹

The ultrastructure of the upper layers of canine atopic skin is also altered. A pilot electron microscopy study reported that the continuity and thickness of the intercellular lipid lamellae were significantly decreased in nonlesional atopic compared with normal canine skin.⁷ A subsequent study in an experimental model evaluating atopic dogs and age- and breed-matched normal dogs before and after allergen challenge (house dust mite) showed that, even before allergen challenge, atopic dogs showed focally severe abnormalities in lipid lamellar organization, with wider intercellular spaces containing abnormal lipid material compared with the compacted, well-organized stratum corneum with regular lipid lamellae seen in the healthy dogs.³⁰ Allergen challenge with house dust mite further worsened the disorganization of the intercellular lipids in atopic dogs, while no appreciable changes were seen in the normal control animals.

Sphingosine-1-phosphate (S1P) is an important component of the stratum corneum lipid milieu. It is both a structural lipid and a signalling molecule that may be involved in modulating altered epidermal lipid profiles.³¹ In mice, S1P also modulates antigen capture by Langerhans cells.³² Alterations in the S1P–S1P-lyase axis may therefore play a role in the pathogenesis of AD. One study found that the mean concentration of S1P in homogenized lesional atopic skin was significantly lower than the levels in skin from healthy dogs.³³ Adding S1P to the epidermal homogenate from lesional atopic skin resulted in significantly higher degradation of S1P. A similar decrease was also found in plasma, suggesting a systemic aberration of the S1P pathway in canine AD.

Filaggrin

Recent studies in human AD have shown that filaggrin mutations are one of the most important risk factors for the development of AD.³⁴ Initial studies using the anti-human filaggrin antibodies in an experimental model of canine AD revealed decreased expression in atopic skin, but use of this reagent was limited by cross-reactivity with other epidermal proteins.³⁵ A further study characterized the unique gene structure of canine filaggrin, which results in production of filaggrin

monomers larger than those of humans or mice.³⁶ This highlights the importance of using antibodies raised against the canine protein. Immunofluorescence microscopy with an antibody targeting the canine filaggrin C-terminus found that a subset of dogs with AD have decreased to undetectable filaggrin expression.³⁷ This missing C-terminus expression is similar to humans, and suggests loss-of-function mutations in the C-terminus portion.³⁷ These findings are corroborated by other studies that reported decreased filaggrin mRNA in the skin of West Highland white terrier dogs with AD.³⁸ Upregulation of *FLG* mRNA in lesional and nonlesional skin of experimentally challenged atopic beagle dogs was confirmed by another study in dogs with naturally occurring AD, which reported an increase in involucrin and filaggrin gene expression in lesional AD skin.^{39,40} The authors reported that this was closely correlated with an increase of proliferative keratins, such as KRT5 and KRT17, and speculated that this may be a response to compensate for abnormal function. It is unclear whether these abnormalities in filaggrin expression are a primary trigger or consequence of AD, and this may vary between dog breeds. It has been suggested that loss-of-function C-terminus mutations play a role in the pathogenesis of canine AD in some breeds, and a single-nucleotide polymorphism in the filaggrin gene was associated with AD in British Labrador retriever dogs.³⁷ However, this was not seen in Labrador retriever dogs from the USA and Japan, or in other breeds in this study.⁴¹ Other studies, furthermore, specifically excluded filaggrin mutations in West Highland white terrier dogs, suggesting that primary alterations in function may be less important in this breed.^{42–44}

In summary, there is considerable evidence associating abnormalities in stratum corneum lipids and proteins with defective epidermal barrier function and clinical canine AD. However, these abnormalities are not consistently found in canine AD patients, and similar defects may be seen in the absence of allergic disease. Similar challenges exist in human medicine, where not all patients with filaggrin mutations develop AD, and AD may exist in individuals without mutations. These findings highlight the fact that AD is a clinical syndrome rather than a single disease and that it can be developed in a variety of ways.

Role of infections in canine atopic dermatitis

The relationship between cutaneous infections and exacerbation of the clinical signs of AD is complex and not completely elucidated. However, many studies in humans have shown a correlation between cutaneous skin infections and severity of AD, suggesting a direct effect of micro-organisms on the immune system. This may also be true in canine AD, although there have been fewer studies and the situation is less clear.⁴⁵

Historical perspective

It was well established in 2001 that dogs with AD frequently demonstrated recurrent bacterial infections of the skin and ears.⁴⁶ In contrast to humans, the species of bacteria most commonly associated with bacterial follicu-

litis was *Staphylococcus intermedius* (now reclassified as *S. pseudintermedius*), although it remained uncertain whether or not this organism was part of the normal cutaneous resident microflora.⁴⁷ Dogs with atopic dermatitis were demonstrated to have increased staphylococcal adherence and colonization of both lesional and nonlesional skin compared with healthy dogs, but the number of bacteria present decreased when dogs went into clinical remission.^{48–50} The relevance of bacterial colonization to the pathogenesis of pruritus was supported by the demonstration that antibiotic treatment alone could improve the clinical signs of AD in many cases.⁴⁶ Indeed, staphylococcal infections could induce cutaneous inflammation and pruritus even in nonatopic dogs.⁴⁶ The mechanism by which this pruritus was induced was still unclear, but it was hypothesized that the production of staphylococcal exotoxins could serve as a source of antigen-nonspecific T-cell stimulation (i.e. superantigens), as had been demonstrated in humans.⁴⁶ Canine isolates of *S. (pseud)intermedius* had been demonstrated to produce exotoxins, but there was no clear evidence to link the production of exotoxins to the clinical characteristics or severity of cutaneous infections in dogs.^{51–53}

Cutaneous infections with yeast (specifically, *Malassezia pachydermatis*), were also known to be increased in dogs with AD.⁴⁶ Intradermal injection with *Malassezia* extracts induced the development of significantly greater wheal-and-flare reactions in atopic dogs with cytological evidence of yeast dermatitis than was seen in atopic dogs without yeast, suggesting that *Malassezia* proteins could act as allergens in atopic dogs.⁵⁴ This hypothesis was supported by the demonstration that dogs with AD, with or without *Malassezia* overgrowth, exhibited higher levels of anti-*Malassezia* IgE than did healthy dogs or nonatopic dogs with *Malassezia* dermatitis.⁵⁵ The ability of *Malassezia* to induce systemic humoral immune responses was also confirmed by the demonstration of significantly higher serum titres of anti-*Malassezia* immunoglobulin G (IgG) and immunoglobulin A (IgA) in dogs suffering from yeast dermatitis compared with healthy dogs.⁵⁶ However, the effects of *Malassezia* upon the development of cell-mediated immunity were less clear. Although one study demonstrated an inhibited lymphocyte blastogenic response to yeast extract in basset hound dogs with yeast dermatitis compared with healthy basset hound dogs, a second study failed to demonstrate a significant difference in blastogenesis between healthy dogs and those with AD.^{56,57}

Update on role of infections in canine atopic dermatitis

The skin microbiome and atopic dermatitis

Recent advances in bioinformatics and high-throughput sequencing technology have revolutionized analysis of the skin microbiome (i.e. the total population of bacteria found at different skin sites). These techniques can identify all the bacteria present to at least genus and usually species level, allowing study of shifts in population dynamics and diversity.⁵⁸ Healthy human skin has a markedly heterogeneous microbiome, with high diversity between individuals and between cutaneous sites. In

contrast, AD is associated with changes in the microbiome that may be relevant to the pathogenesis of the condition. In a recent study, 16S ribosomal RNA bacterial gene sequencing was performed on DNA from skin samples of children with AD.^{59,60} Microbial diversity was markedly reduced during flares of their AD. However, patients with recent nonantimicrobial therapy, even intermittent treatment, showed greater diversity during flares than those with no recent treatment. Skin barrier care and anti-inflammatory treatment appeared to increase the diversity of the skin bacterial flora. Of particular importance, flares were associated with increasing dominance of the flora by staphylococci, especially *S. aureus*. This was correlated with the clinical severity. In contrast, the proportion of *Streptococcus*, *Propionibacterium* and *Corynebacterium* species increased following treatment.

Studies of the canine and feline skin microbiome are much more limited. Studies have tended to focus on a relatively small number of species, suggesting that the cutaneous bacterial flora has low abundance and diversity, but this probably reflects technical limitations.⁶⁰ However, newer, more sophisticated methods may overcome these limitations. In one recent study, bacterial identification was performed using 454-pyrosequencing of the 16S rRNA gene.⁶¹ This work demonstrated a much more diverse cutaneous microflora than had been described previously. Interestingly, fewer bacterial species were identified in samples from dogs with AD, suggesting that the decreased microbial diversity seen in humans with AD may also be present in affected dogs.

Staphylococcal colonization and diversity in atopic dogs

Recurrent staphylococcal skin and ear infections are common in dogs with AD.⁴⁶ This is probably related to more frequent bacterial colonization of atopic compared with healthy skin. It has recently been shown that up to 91.7% of dogs with AD are colonized by a heterogenic population of coagulase-positive staphylococci (90% *S. pseudintermedius* and 4% *S. aureus*) compared with only 39.5% of healthy dogs (84% *S. pseudintermedius* and 8% *S. aureus*).^{62,63} The cornified layer of atopic skin harbours more bacteria than in healthy dogs, although this difference disappears when atopic dogs are in remission (following glucocorticoid therapy).^{50,64} *Staphylococcus pseudintermedius* adheres more readily to both nonlesional and lesional atopic canine skin compared with healthy skin.^{49,65,66} Interestingly, adherence was significantly greater in dogs with high pruritus scores, but was not related to the presence or history of pyoderma.⁶⁶ Other results suggest that adhesion of *S. pseudintermedius* to canine corneocytes varies with breed and body site, but these differences did not account for the predilection sites of bacterial pyoderma.⁶⁷ Atopic and healthy dogs are colonized by a wide variety of distinct isolates of *S. pseudintermedius* that belong to at least nine clonal groups, but none of these was specifically associated with atopic skin, healthy skin or cases of pyoderma.^{62,63} Staphylococcal isolates from atopic and healthy skin, furthermore, exhibited identical adhesion to fibronectin, cytokeratin and fibrinogen *in vitro*.⁶⁸ These molecules (along with P-selectin) can be expressed on the skin surface and stratum corneum in inflamed skin, suggesting

that colonization and infection of atopic skin is associated with host factors and the local environment rather than specific bacterial virulence factors.⁶⁹

Staphylococcus-mediated inflammation in AD

Staphylococcal proteins can penetrate the disrupted stratum corneum following mast cell degranulation, leading to a stimulation of the immune response in both humans and dogs.^{48,70} This effect does not require the development of tissue invasion or folliculitis, as antibiotic-responsive erythema and pruritus have been reported in association with surface bacterial overgrowth in both allergic and nonallergic dogs.⁷¹ In atopic humans, specific IgE, immediate and late phase skin reactivity, positive patch tests, lymphocyte proliferation tests, production of superantigens and exacerbation of the atopic lesions by staphylococci have all been demonstrated.^{72,73} Exacerbation of human AD by *S. aureus*, furthermore, is associated both with specific IgE and superantigens. Specific IgE antibodies against *S. pseudintermedius* have been detected in canine AD, but their role is unclear.⁷⁴ Likewise, one study showed an increase in total serum IgG in dogs with AD with superficial pyoderma, but a decrease in total serum IgA in dogs with AD without pyoderma.⁷⁵ In addition, an increase in antistaphylococcal IgG but not IgA was seen in dogs with AD with or without pyoderma when compared with healthy control animals.⁷⁵ More recently, increased levels of antistaphylococcal IgE were found in atopic dogs compared with nonatopic dogs with pyoderma, healthy dogs and specific pathogen-free dogs.⁷⁶ Levels of antistaphylococcal IgG, in contrast, were comparable in the atopic and nonatopic dogs.

Some staphylococci are able to produce toxins that can alter the skin barrier, stimulate the immune system and trigger atopic lesions in humans. These include enterotoxins and exfoliative exotoxins produced by *S. aureus*, *S. pseudintermedius* and *S. hyicus*.^{73,77–79} These molecules act as superantigens, which are able to bypass the normal control of T-cell activation and trigger a massive stimulation of large numbers of T cells, leading to vigorous T-cell activation and cytokine release.^{80,81} Staphylococcal superantigens in humans activate keratinocytes, induce cutaneous leucocyte antigen expression on T cells, induce major histocompatibility complex II, interleukin-1, tumour necrosis factor- α , interleukin-4 and interleukin-12 expression by mononuclear cells, decrease IFN γ production and upregulate endothelial intercellular adhesion molecule-1 and vascular adhesion molecule-1.^{82–86} Finally, superantigens are able to stimulate *Staphylococcus*-specific IgE, which is able to bind to mast cells or Langerhans cells. This exacerbates the clinical signs of AD in humans through recirculation of activated T cells to the skin and on-going inflammation.^{87–91}

Up to 25% of canine strains of *S. pseudintermedius* are able to produce superantigens and stimulate canine peripheral blood mononuclear cell proliferation.⁸⁰ However, although up to 40% of *S. pseudintermedius* strains are able to produce exotoxins, a correlation between exotoxin production and the types of lesions or severity of pruritus in canine AD was not found.^{51–53} Canine keratinocytes produce thymic stromal lymphopoietin, which

appears to be important in activating Langerhans cells and inducing inflammation.⁹² Culture of canine keratinocytes with Toll-like receptor (TLR) 3 or TLR4 ligands increased thymic stromal lymphopoietin expression, while exposure to TLR2 or TLR7 ligands did not. Toll-like receptor 3 and TLR4 both bind microbial antigens (double-stranded RNA and lipopolysaccharide), and this may be one explanation for exacerbation of cutaneous inflammation by microbial colonization.

It is likely that the immunological effects seen in human AD also occur in dogs, but more studies are necessary to confirm the role of staphylococcal colonization and infection in canine AD. Atopic dogs with recurrent staphylococcal infections do benefit from regular topical or systemic antimicrobial therapy, although a specific hypersensitivity has not always been demonstrated.⁹³ However, with recent concerns over antimicrobial resistance, routine pulse therapy with topical or systemic antibiotics may be unwise; treatment should instead focus on topical antimicrobials and control of the underlying inflammation.^{94–96}

Bacterial resistance and AD

The prevalence of bacterial resistance has increased in both human and veterinary medicine.^{96–102} The conventional treatments used for recurrent pyoderma in dogs with AD may represent a potential risk for an increase of resistance in the future. Several studies have shown that human AD patients are more susceptible to methicillin- or multidrug-resistant *S. aureus* when compared with nonatopic patients.^{97–99} In particular, up to 60% of atopic children with skin infections were colonized by methicillin-resistant *S. aureus* compared with only 30% of atopic children without skin infections.⁹⁹ No comparable figures for canine AD exist, but this is a concern given the prevalence of secondary staphylococcal infections in these patients, frequent antibiotic use and the increasing prevalence of methicillin- or multidrug-resistant *S. pseudintermedius* in veterinary practice.¹⁰⁰

Malassezia colonization and diversity in atopic dogs

Malassezia skin and ear infections are also common in canine AD.⁴⁶ Only one study has been published on the occurrence of *Malassezia* spp. in atopic versus healthy dogs.¹⁰³ This study demonstrated the more frequent occurrence of *Malassezia* spp. in atopic dogs compared with healthy control animals. In another study, it was reported that 100% of dogs with AD are colonized by *M. pachydermatis*, with the interdigital areas (70.7%) and ears (63.4%) the most commonly involved sites.¹⁰⁴ However, the study did not include a healthy control population. A more recent study showed no difference in the frequency of *M. pachydermatis* colonization in ears of atopic (33.3%) versus healthy dogs (21%).¹⁰⁵ Finally, one study showed that while *Malassezia* isolates isolated from healthy skin were very diverse, an almost monogenic population was present on atopic skin.¹⁰⁶ Isotype 3D, which was recovered from 91% of the atopic dogs, had the highest production of phospholipase A2 and was more active than other isolates at higher pH, suggesting a possible relationship in the development of canine AD.¹⁰⁶ Phospholipase A2 is of

particular interest because it may disrupt the epidermal barrier.

These findings suggest that looking at colonization rates in canine AD may be misleading, because it is likely that most if not all dogs are colonized with *Malassezia*. It may be more relevant to look at density, population heterogeneity and the frequency of infection. It is likely that many of the same factors favouring staphylococcal colonization and infection play a similar role in *Malassezia* dermatitis and otitis. There are some differences between *Malassezia* isolates from atopic and healthy skin, but overall it is likely that host factors are more important than microbial virulence factors.

Malassezia spp. and AD

The involvement of *Malassezia* in the pathogenesis and exacerbation of human and canine AD has been well demonstrated.^{46,107,108} It is likely that most of the virulence factors analysed above for bacteria (such as the increased adherence to atopic keratinocytes) play a similar role in *Malassezia* colonization and infection. In particular, production of phospholipase A2 (which is able to disrupt a host's cell membranes) is a possible initiating factor for *Malassezia* dermatitis secondary to AD in dogs.^{106,109,110}

There is a strong relationship between *Malassezia* spp. and the host's immune system in both human and canine AD. In particular, *Malassezia* spp. are able to induce interleukin-1 β , interleukin-6, interleukin-8 and tumour necrosis factor- α production in normal human keratinocytes.¹¹¹ *Malassezia* colonization is associated with high levels of serum specific IgE, immediate and late phase skin reactions, positive patch tests and increased peripheral blood mononuclear cell proliferation in humans with AD but not in healthy subjects.^{112–114} Finally, major and minor *Malassezia* antigens (varying in size from 10 to 100 kDa depending on the species) have been identified in human AD.^{108,110,114} One major 37 kDa allergen, Mala f 1, induces histamine release from human basophils, but there is no evidence of superantigen activity.

Similar to humans, serum specific IgE, positive intradermal test reactivity, positive passive transfer reactions and stimulation of peripheral blood mononuclear cell proliferation have been demonstrated in atopic dogs, showing that *M. pachydermatis* acts as a conventional allergen in canine AD.^{54,115–117} Furthermore, multiple major (45, 52, 56 and 63 kDa proteins) and minor antigens have been recognized by serum IgG and IgE in canine AD.¹¹⁸ Despite this, crude *M. pachydermatis* extracts failed to induce keratinocyte proliferation in explant cultures of canine skin.

It therefore appears that *Malassezia* may contribute both directly (by the induction of inflammatory cytokines) and indirectly (by acting as an allergen) to the inflammation associated with canine AD. Anecdotal evidence suggests that dogs with positive *Malassezia*-specific intradermal tests or serology may benefit from regular topical or systemic anti-*Malassezia* therapy, although randomized controlled clinical trials have not been performed. It is not clear whether allergen-specific immunotherapy with *Malassezia* extracts is effective.

Antimicrobial peptides and AD

Antimicrobial peptides (AMPs), particularly β -defensins (BDs) and cathelicidins (Caths), may play an important role in skin barrier function and innate immunity in human and canine AD.^{119–121} These peptides have two main functions; they have direct antimicrobial activity against a variety of micro-organisms and they modulate innate and adaptive immune responses.^{120,122–124} More recently, it has become clear that BDs are also involved in many other functions, including cell recruitment and activation, wound healing and coat colour in dogs. Expression in inflammatory diseases is complex and variable; a causal association with AD has become less clear.

The relationship between AMPs and skin infections in AD has been extensively studied in humans but has only been investigated in dogs recently. Alterations in expression of several AMPs have been demonstrated in human AD, suggesting a complex interaction between AMPs, atopic disease and cutaneous microflora. Human AD has been associated with altered regulation of BDs and other antimicrobial peptides, including single-nucleotide polymorphisms in the gene for hBD1. hBD3 is thought to be the most important in defence against staphylococci. It is not constitutively expressed in human skin, but is induced by tumour necrosis factor- α and other inflammatory mediators, bacteria and bacterial products. Increased mRNA and protein expression of hBD2, hBD3, RNase7 and psoriasin is present in lesional atopic skin when compared with healthy control subjects.^{125–131} This was even more evident in the skin of patients suffering from the extrinsic form of AD or after tape stripping.^{128,130} On the contrary, no difference was observed in nonlesional skin when compared with healthy control subjects.^{129,132} Expression of human Cath (LL-37) was comparable in lesional atopic and healthy skin,^{126,127,133} while expression of dermcidin was decreased in atopic compared with healthy patients.¹³⁴ Despite these findings, no correlation between increased RNase, hBD2 and psoriasin expression, *S. aureus* colonization and the severity of human AD has been found.⁹⁹

Fewer studies have been published on the possible involvement of AMPs in the pathogenesis of canine AD. In naturally affected atopic dogs (treated for bacterial and yeast infections prior to inclusion in the study), there was an increase in mRNA expression of cBD1 (12-fold in lesional and 5-fold in nonlesional atopic skin) and S1008A (390-fold in lesional and 15-fold in nonlesional skin) and a decrease in cBD103 (the canine orthologue of hBD3; 2.5-fold in nonlesional skin).¹³⁵ Those data were not confirmed by others, who showed an increase in cBD103 (1.8-fold) in older naturally affected dogs without previous antimicrobial treatments.¹³⁶ Expression of cBD103 was greater in actively infected skin when compared with atopic skin without active infection (3.8-fold) or with healthy control skin (4.4-fold).¹³⁶ In the same study, decreased expression of cBD1-like (0.5-fold) was also seen in infected atopic skin when compared with noninfected atopic skin.¹³⁶ Finally, dogs from a colony of experimentally induced atopic beagle dogs had an increase in mRNA expression of cBD1-like (9.5-fold in lesional and 10-fold in nonlesional skin), cBD3-like (9-fold in lesional and 6-fold in nonlesional skin) and cCath (6.5-fold in lesional and 5-fold

in nonlesional skin) when compared with healthy breed-matched dogs.¹³⁷ In contrast, other studies have shown similar cBD103 expression levels in lesional atopic, nonlesional atopic and healthy skin, and infected atopic, noninfected atopic and healthy skin.^{138,139}

The role of AMPs in the pathogenesis of canine AD is therefore still unclear and requires further study. No correlation between AMP expression and staphylococcal colonization or clinical severity has been consistently established. It is likely that there is a complex relationship between AMPs, the cutaneous microflora and AD, which may vary with the breed, genotype and clinical phenotype.

Conclusions and recommendations for future studies

The past 12 years have been particularly productive in veterinary dermatology, especially in the development of improved research technology and methods. Such new and improved methodologies and technologies have opened more possibilities of identifying the primary culprits involved in the pathogenesis of canine AD.

An exciting development over the past 12 years has been the greater understanding of the role of the epidermal barrier function in the pathogenesis of canine AD. Defects or dysfunction of individual components of the epidermis and stratum corneum have long been appreciated as the cause of certain defined skin diseases (such as ichthyosis). More recent evidence suggests that far more subtle changes in some factors may be associated with the perpetuation and development of canine AD. The most well known of these is filaggrin, which can be abnormally expressed in the skin of dogs with AD.^{37–40} Evidence suggests that canine AD may also be associated with a disruption in epidermal lipid production and function.^{7,22,26} However, it remains unclear whether these abnormalities are actively involved in the development of sensitization and clinical disease or if they amplify changes that are already present. Prospective, case-controlled studies to compare epidermal structure and function in allergy-predisposed and related control dogs would provide a wealth of information regarding the role of the epidermal barrier in the development of canine AD. Many dogs with AD may benefit from skin barrier care, but well-conducted and -designed clinical trials are needed to determine the efficacy of products before they can be recommended specifically. Research may also identify dogs with primary skin barrier defects where early life interventions may prevent or ameliorate the later development of AD.

Of major importance has been the identification of host–environmental interactions, highlighted by new information demonstrating that surface microbes may not only invade and infect the skin of atopic patients but can contribute directly to the inflammatory response as conventional allergens or by superantigen-mediated polyclonal activation of T cells and stimulation of cutaneous inflammation.^{54,75,80,115,118} Understanding of the potential for microbes as ‘flare factors’ for canine AD has driven the development of treatment strategies designed to minimize cutaneous colonization with bacteria and yeast organisms.

In conclusion, the past 12 years have provided a wealth of information regarding the factors (internal and external) that play a role in the development and perpetuation of canine AD. This information is vital not only for characterization of atopic responses but also for the development of novel, practical and evidence-based therapeutic approaches to patient management.

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Résumé

Contexte – La dermatite atopique canine (AD) est une dermatite prurigineuse, inflammatoire, génétique-ment prédisposée et fréquente. La pathogénie de l'AD canine est incomplètement comprise.

Objectifs – Le but de cette revue était de fournir une mise à jour approfondie de l'implication de la barrière cutanée et de l'interaction avec le microbiome de l'hôte dans la pathogénie de l'AD.

Méthodes – Les citations des bases de données en ligne et les résumés des congrès internationaux liées à la barrière cutanée et à l'interaction avec le microbiome de l'hôte (e.g. bactéries, levures, peptides antimicrobiens) ont été recherchées.

Résultats – Un total de 126 publications ont été identifiées. Cette revue se concentre sur les dysfonctions de la barrière épidermique et l'interaction entre les microbes cutanés (bactéries et levures) et l'hôte (peptides antimicrobiens). Les nouveautés épidémiologiques sur la présence d'organismes pathogéniques et l'AD canine ont aussi été fournies.

Conclusions et importance clinique – Des avancées majeures ont été faites sur l'investigation des dysfonctions de la barrière cutanée de l'AD bien que de nombreuses questions restent en suspens. Les interactions entre microbiome-hôte et les dysfonctions de la barrière cutanée apparaissent comme des altérations primaires de l'AD canine. A partir de cette revue, il semble clair que des études futures concernant le développement de traitements capables de restaurer la barrière cutanée et d'augmenter les défenses naturelles contre les organismes pathogéniques soient nécessaires.

Resumen

Introducción – la dermatitis atópica (AD) es una enfermedad inflamatoria y pruriginosa de la piel bastante común y con predisposición genética. Su patogenia no está bien clarificada.

Objetivos – el propósito de esta revisión es aportar una puesta al día muy completa del papel de la barrera de la piel y la interacción de la microflora con el hospedador en la patogénesis de la AD canina.

Métodos – se buscaron citas online y resúmenes en bases de datos de congresos internacionales relacionados con la barrera de la piel y la interacción microflora-hospedador (por ejemplo bacterias, levaduras, péptidos antimicrobianos).

Resultados – se identificaron un total de 126 publicaciones. Esta revisión se centra en anomalías de la función de barrera y la interacción entre los microbios cutáneos (bacterias y levaduras) y el hospedador (péptidos antimicrobianos). También se aportan nuevos resultados epidemiológicos de la presencia de organismos patógenos en la AD canina.

Conclusiones e importancia clínica – se han realizado grandes avances en la investigación de las anomalías en la función de barrera de la piel, aunque aún quedan muchas cuestiones sin resolver. Las anomalías de la función de barrera de la piel y las interacciones microflora-hospedador están surgiendo como alteraciones primarias en la AD canina. Basados en esta revisión está claro que se necesitan estudios basados en el desarrollo de fármacos capaces de restaurar la barrera de la piel e incrementar la defensas naturales frente a organismos patógenos.

Zusammenfassung

Hintergrund – Die atopische Dermatitis (AD) des Hundes ist eine häufige, genetisch prädisponierte, entzündliche und juckende Hauterkrankung des Hundes. Die Pathogenese der AD des Hundes ist nur unvollständig bekannt.

Ziele – Das Ziel dieser Review ist es, ein umfassendes Update über die Beteiligung der Hautbarriere und der Interaktionen zwischen Wirt und Mikrobiom bei der Pathogenese der caninen AD zu erstellen.

Methoden – Es wurden online Datenbasen und Abstracts von internationalen Meetings auf Publikationen, die mit der Hautbarriere und der Wirt-Mikrobiom Interaktion (z.B. Bakterien, Hefen, antimikrobielle Peptide) im Zusammenhang standen, durchforstet.

Ergebnisse – Insgesamt wurden 126 Publikationen gefunden. Dieser Review Artikel konzentriert sich in erster Linie auf die Dysfunktion der epidermalen Barriere und die Interaktion zwischen den Hautmikroben (Bakterien und Hefen) und dem Wirt (antimikrobielle Peptide). Epidemiologische Updates über das Vorkommen pathogener Organismen und der AD des Hundes wurden ebenfalls bereitgestellt.

Schlussfolgerungen und klinische Bedeutung – Großartige Fortschritte sind bei der Untersuchung der Hautbarrierendysfunktion bei der AD des Hundes gemacht worden, obwohl noch immer viele Fragen offen

sind. Die Dysfunktion der Hautbarriere und die Wirt-Mikrobiom Interaktionen treten als primäre Veränderungen bei der AD des Hundes hervor. Basierend auf dieser Review ist klar, dass zukünftige Studien, die sich auf die Wiederherstellung der Hautbarriere und der Zunahme der natürlichen Abwehrmechanismen gegen pathogene Organismen konzentrieren, nötig sind.

要約

背景 — イヌアトピー性皮膚炎(AD)は遺伝的に好発する一般的な、炎症性および掻痒性皮膚疾患である。イヌADの病因は完全には理解されていない。

目的 — この総説の目的はイヌのADの病因における皮膚バリアや宿主の微生物叢に関する広範囲にわたる最新情報を提供することである。

方法 — オンライン引用データベースおよび国際的な会議での要約に対して皮膚バリアや宿主-細菌叢相互反応(例として、細菌、酵母菌)についての出版物の検索を行った。

結果 — 126の公表文献を確認した。この総説記事は表皮バリア機能不全、皮膚微生物(細菌と酵母菌)の間の相互反応、および宿主(抗菌ペプチド)に焦点をあてた。病原性微生物の存在とイヌADの疫学的なアップデートも行った。

結論および臨床的な重要性 — 多くの疑問が未だ残っているが、イヌADにおける皮膚バリア機能不全の研究として、重要な進歩をとげた。皮膚バリア機能不全と宿主-細菌叢の相互作用はイヌADにおいて、一時的な変化として生じることが明らかになった。この総説に基づくと、皮膚バリアを回復させ、病原性微生物に対する自然防御を高める薬剤の開発に焦点を当てた今後の研究が必要であることが明らかである。

摘要

背景 — 犬异位性皮膚炎(AD)は種常見的、遺伝性、炎性及掻痒性皮膚病。犬ADの发病机理还未完全明确。

目的 — 本次研究的目的是,关于犬AD发病机理,在皮肤屏障和宿主-微生物互作方面,提供深入更新。

方法 — 从国际会议上搜索有关皮肤屏障和宿主-微生物互作(如细菌、酵母菌、抗生物肽)的出版物,其在线引文数据库和摘要。

结果 — 总共有126篇文献。这些文章聚焦于表皮屏障功能障碍以及皮肤微生物(细菌和酵母菌)和宿主(抗生物肽)的互作。更新了病原微生物和犬AD流行病学。

总结与临床意义 — 主要进展为调查犬AD的皮肤屏障功能障碍,虽然很多问题仍未解决。皮肤屏障功能障碍及宿主-微生物相互作用是犬AD的主要改变。基于这些研究,未来实验将集中开发能还原皮肤屏障,以及增加对抗致病菌自然防御力的药物上。