Reference Point

Current understanding of the pathophysiologic mechanisms of canine atopic dermatitis

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Our thinking about the skin disease in dogs currently called AD has undergone immense changes in the past 75 years. First called eczema in dogs by Schnelle in 1933, it was later termed canine allergic inhalant dermatitis and then canine atopy. The skin disease associated with atopy in dogs is now referred to as canine AD.

Since the first description of this condition, huge strides have been made in human medicine, particularly in the biomedical sciences, with regard to how the immune system works. Basic scientific knowledge has crossed over into the field of clinical medicine, resulting in improved patient care. Much of what is understood about allergies in humans can be applied to veterinary patients, with the caveat that dogs are not humans. Nevertheless, although there are species differences in some of the aspects of immune function among species, mammalian immune function is fairly consistent and some of the principles understood in human medicine can be applied to veterinary patients, always being careful to verify via research and clinical studies that the pathophysiologic mechanisms in humans hold true for species of interest in veterinary medicine. To understand the current knowledge on the pathophysiologic mechanisms of canine AD, preconceived notions must be put aside and the newer evidence-based information examined.

Where We Started

Eczema in dogs was first described in 1933 by Schnelle. Dogs were tested by a scratch test with 12 commercially prepared food allergens. When the dogs were challenged by being fed foods to which they had reacted, they seemed to be more pruritic.

Later that same year, Burns published a report describing more extensive testing with 21 food extracts on 25 dogs. After a negative scratch test result, an intradermal test with food extracts was performed on 65 dogs. Five dogs were fed the food to which they had tested positive. Only 3 reacted with gastrointestinal disturbances, and only 1 dog developed a cutaneous reaction.

In 1934, Pomeroy intradermally tested 76 dogs with and without skin lesions. Positive reactions were seen in 19 of 58 (33%) dogs without skin lesions and 9 of 18 (50%) dogs with skin lesions. All of these early cases of dogs with allergic eczema were believed to be caused by foods alone.

In 1941, Wittich, a physician, published a case report of a dog that had a 6-year history of signs of seasonal conjunctivitis, rhinitis, facial urticaria, and pruritus, but not what is typically referred to now as the classic clinical signs of AD in dogs. The clinical signs resolved when the dog was moved to a different pollen-free environment but recurred when returning to its home environment. This dog had possibly inhaled environmental allergens (weed pollens), which may have been the cause of the seasonal allergic rhinitis (hay fever) because the animal reacted to ophthalmic and intranasal challenges with ragweed pollens. Wittich termed the condition atopy. After intradermal testing to identify the offending allergens, allergen-specific immunotherapy was performed and the clinical signs did not recur the following year.

Eighteen years later, in 1959, Patterson, also a physician, published the first articles in the veterinary literature on ragweed allergy in a dog. Again, the primary clinical signs involved the upper respiratory tract, consisting primarily of lacrimation and conjunctivitis. The dog was pruritic and had an erythematous and scaly dermatitis on the flexural surface of the forelegs and on the back. Following inhalant challenge with the offending allergens that had been identified with an intradermal test, the respiratory signs worsened and included those of asthma. The dog also developed vomiting and diarrhea consistent with anaphylaxis after IV challenge with the pollen extract. Conjunctival testing with ragweed pollen was positive. These reports do not mention any signs of AD or pruritus in the dog at the time of challenge with the ragweed or pollen allergens.

Abbreviations

| AD | Atopic dermatitis |
| IL | Interleukin |
| LT | Leukotriene |
| Th1 | T-helper type 1 cell |
| Th2 | T-helper type 2 cell |
Patterson and Sparks\(^8\) followed by Patterson et al\(^9\) went on to show that this same dog with ragweed hypersensitivity had a serum antibody with the same characteristics as human skin-sensitizing antibody from patients with pollen allergy. Again, the clinical signs noted in these reports\(^8,9\) were signs of asthma and anaphylaxis, not AD, and were confirmed with a positive intradermal test result.

All of these observations supported the fact that dogs could produce an allergen-specific antibody after aerosolized allergen exposure to pollens and that with reexposure, clinical signs consistent with atopic conjunctivitis, rhinitis, asthma, and anaphylaxis could occur. Skin sensitivity could be confirmed with a positive intradermal test, but none of the dogs described in these early reports had classical clinical signs of AD. The clinical signs described were all consistent with what is known to be a type I hypersensitivity reaction: asthma, conjunctivitis, urticaria, pruritus, anaphylaxis, and possibly angioedema.

From these early descriptions of atopic disease in dogs, in 1965, Schwartzman\(^10\) began to group together dogs with signs of allergic upper respiratory disease and pruritus under the diagnosis of atopy. A simple pathophysiologic mechanism of the pruritic skin disease was accepted. Dogs would become sensitized to environmental allergens either by the respiratory route or by percutaneous absorption. This would trigger the production of allergen-specific IgE antibodies. These antibodies would bind to mast cells and basophils in the dermis. Reexposure to the offending allergen would result in the degranulation of the mast cell or basophil and the release of their contents, such as histamine, serotonin, and eosinophil chemotactic factor. These inflammatory cytokines caused the erythema and pruritus seen clinically in affected dogs. Atopic disease in dogs then became a disease that dermatologists diagnosed and treated, and if a respiratory component existed, it was considered an incidental finding. This simplified explanation of the mechanism of canine atopy remained the accepted dogma for many years (Figure 1).

**Where We Are Now**

**Role of IgE and histamine in AD in dogs and humans**—Canine IgE was first described in the 1970s and was shown to have properties similar to human IgE.\(^11,12\) In 1973, Halliwell\(^13\) was the first to report on IgE in the skin of clinically normal dogs. Immunoglobulin E was shown to be localized to the surface of cutaneous mast cells in canine skin, suggesting its involvement in the pathogenesis of canine AD.\(^13\) Several years later, the physiochemical properties of canine IgE were further elucidated.\(^11\)

Subsequent to these reports, intradermal testing of pruritic dogs with a wide variety of antigens revealed that dogs reacted to intradermal tests involving numerous allergenic extracts, including house dust; pollens from trees, weeds, and grasses; epidermal antigens; and miscellaneous antigens from various foods and kapok.\(^14–22\)

Some of the dogs included in these reports had clinical signs consistent with AD, whereas others were examined for clinical signs of seasonal allergic rhinitis or asthma. Unfortunately, it was not until 1982 when August\(^23\) intradermally tested 90 dogs with no clinical signs of AD with 6 dilutions of 25 allergenic extracts, including house dust, 7 foods, 2 mold mixtures, a variety of tree and weed pollens, and miscellaneous allergens.\(^23\) That study demonstrated positive intradermal test reactivity to house dust mite allergens in 90% of the dogs at a standard intradermal testing concentration (1,500 protein nitrogen units/mL) and in 60% of the dogs at the lowest concentration (250 protein nitrogen units/mL). Testing with several of the other allergens also resulted in positive reactions at the lowest test concentration. This study confirmed that subclinical reactions could occur in clinically normal dogs. In 1995, Codner and Tinker\(^24\) showed that 50% to 58% of clinically normal dogs had positive intradermal test results to house dust mites (1:5,000 wt/vol) and house dust (100 protein nitrogen units/mL) when tested as per the manufacturer’s recommendation. These results were reported about 40 years after the term atopy was used in the veterinary literature and about 20 years after intradermal testing was used routinely as a means to diagnose AD in dogs.

![Figure 1—Depiction of the historically accepted pathophysiologic mechanism of canine AD.](image-url)
Even more interestingly, it is now recognized that there are dogs (and people) with AD for which no allergen-specific IgE can be identified with either intradermal testing or allergen-specific IgE serologic testing directed against common allergens. One study documented this fact in 21 of 82 (26%) consecutive dogs with AD. These findings raise the possibilities that these dogs were not allergic or they were not allergic to routinely tested allergens. In human medicine, the terms atopic-like dermatitis, intrinsic AD, or nonallergic AD have been used to describe a subset of patients (approx 10%) in which classic clinical signs of AD are present but no circulating nor cutaneous allergen-specific IgE can be demonstrated against major allergens. In some of these human patients, it is speculated that the absence of detectable allergen-specific IgE may represent the early stages of AD in which allergenic sensitization has not yet occurred. In other individuals, however, it appears that AD can occur through alternative pathways that are not IgE dependent. It can also be speculated that these people could be reactive to a group of minor allergens that remain unidentified. Whether the same applies to dogs is unknown at this time.

It is also important to point out that allergen-specific IgE may be detected in dogs that do not have clinical manifestations of AD and that measurement of IgE does not help to discriminate between dogs that will later develop disease and dogs that will not. Similarly, measurement of IgE is not helpful in discriminating between atopic dogs, clinically normal dogs, and dogs with parasitic diseases. It may be speculated that not all IgE is pathogenic. Altogether, these considerations highlight the fact that the initial view of AD as solely an IgE-mediated allergic disease does not completely explain the pathogenesis of this complex disease and that new definitions and approaches are needed.

Definitions of atopy, AD, and atopic-like dermatitis in dogs and humans—In 2001, the American College of Veterinary Medicine Task Force on Canine Atopic Dermatitis accepted the definition of atopy as “a genetically predisposed tendency to develop IgE-mediated allergy to environmental allergens.” The environmental proteins to which the body overreacts or reacts in an abnormal manner are called allergens and include pollens, molds, dusts, danders, mites, and, in some cases, insects, chemicals, and foods. The pathogenesis of atopy is mediated by numerous genetic as well as environmental factors. Patients prone to IgE-mediated allergic reactions are said to be atopic, and the condition is classically defined as a type I hypersensitivity. In humans, atopy comprises the clinical triad of seasonal allergic rhinitis, asthma, and AD, whereas in dogs, atopic seasonal allergic rhinitis or asthma is rarely diagnosed.

As per the International Task Force on Canine Atopic Dermatitis in 2006, the currently accepted definition for canine AD (previously known as allergic inhalant dermatitis, seasonal allergy, environmental allergy, and atopy) is a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features associated with IgE antibodies most commonly directed against environmental allergens. This is referred to as the extrinsic form of AD. Canine atopic-like dermatitis has also been recognized and defined as an inflammatory and pruritic skin disease with clinical features identical to those seen in canine AD, in which an IgE response to environmental or other allergens cannot be documented. Lack of documentation of allergen-specific IgE does not imply that environmental or other allergens are not involved in the pathogenesis, but simply that allergen-specific IgE has not been identified against the common allergens by intraderal or serologic testing. Some equate this with what is now called the intrinsic form of the disease.

Atopic dermatitis in humans is poorly defined and is described as an immune-mediated inflammation of the skin arising from an interaction between genetic and environmental factors. Findings of 1 study indicate that a heritable epidermal barrier defect is a primary cause of AD in humans; a defect in the filaggrin gene is specifically implicated as one of the most important risk factors. Erythema and pruritus are the primary clinical signs in humans; skin lesions range from mild erythema to severe lichenification. Intrinsic AD (70% to 80% of cases) occurs when environmental exposures trigger immunologic, usually allergic (ie, IgE-mediated) reactions in genetically susceptible people. Intrinsic AD is nonfamilial and idiopathic, and its pathophysiologic features are generally not well understood. In human medicine, it is currently suggested that the intrinsic form of AD may represent the initial step of AD, before any allergic sensitization has occurred yet.

Understanding AD in dogs—It is now accepted that canine AD, similar to the human counterpart, is a multifaceted disease determined by a combination of genetic and environmental factors affecting both the immunologic response as well the skin barrier function to be either a primary or secondary aspect.

Involvement of IgE and other reaginic antibodies

Most of the initial research emphasis had been placed on the humoral response (mostly IgE) to allergens. This view affected both the definition of this disease (as a type I hypersensitivity with consequent mast cell degranulation) and one of the most commonly used treatment options (eg, antihistamines). After the initial discovery of canine IgE, extensive subsequent research demonstrated the presence of allergen-specific IgE in dogs with AD and involved the use of both intraderal tests and in vitro allergen-specific IgE assays. Although IgE may be involved in the pathogenesis of most cases of canine AD, the development of the disease is likely to be dependent on a range of other factors that may include T-cell subpopulation polarization, altered mast cell releasability, and defective barrier function. A role for allergen-specific IgGd in the pathogenesis of canine AD has also been proposed, but this is now regarded as controversial by other authors. As research progressed, it was clear that IgE was only a piece of the puzzle and that although IgE plays an important role in AD in both the allergic phase and the elicitation phase, IgE per se...
Histamine was a key mediator, a study in which plasma LTB4 concentrations in dogs with AD were measured and found to be high in all of these conditions. A positive correlation between LTB4 concentrations and the degree of pruritus was found in that study. The lack of specificity of LT measurement in the diagnosis of canine AD does not lessen the pathogenic implications of these findings because different diseases may trigger similar inflammatory responses.

In a pilot study, sulphido-LT production in the skin of dogs with AD and healthy control dogs was evaluated after stimulation with saline (0.9% NaCl) solution, lipopolysaccharide, and a reference allergen. No significant difference in sulphido-LT production was found between groups in response to any of the stimuli. No difference in sulphido-LT production was detected in the skin between clinically normal dogs and dogs with AD with no skin lesions; similarly, no difference in sulphido-LT production was found between dogs with AD that did and did not have skin lesions. Although preliminary, these results do not support a role for sulphido-LT in canine AD.

By contrast, the importance of LT in the pathogenesis of AD is suggested by the possible beneficial
cannot explain all aspects of this complex disease. Although it is currently accepted that IgE amplifies and increases the efficiency in capturing the allergen as well as participates in the inflammatory response that results, AD can manifest in the absence of detectable allergen-specific IgE.

**TYPE I HYPERSENSITIVITY**

Type I allergic reactions manifest clinically as anaphylaxis, allergic asthma, urticaria, angioedema, allergic rhinitis, some types of drug reactions, and AD. These reactions tend to be mediated by IgE, which differentiates them from anaphylactoid reactions that involve IgE-independent mast cell and basophil degranulation. Interestingly, the most classic type I hypersensitivity reactions (anaphylaxis, asthma) are not pruritic, although other conditions such as allergic urticaria and rhinitis can be. Histamine is one of the preformed molecules produced in high concentrations by mast cells and basophils found in nearby connective tissues. Its major function is to cause vasodilatation, and it also triggers the inflammatory response. As part of an immune response to foreign pathogens, histamine is released. It increases the permeability of the capillaries to WBCs and other proteins to allow them to engage foreign invaders in the affected tissues.

H1 histamine receptors are found on smooth muscle, endothelium, and CNS tissue. Binding of histamine causes vasodilatation, bronchoconstriction, bronchial smooth muscle contraction, separation of endothelial cells (responsible for hives), and pain and itching in response to an insect sting. H1 histamine receptors are the primary receptors involved in the clinical signs of allergic rhinitis and motion sickness.

The importance of histamine as a major mediator of pruritus in dogs with AD is controversial. Although the traditional view of AD would have suggested that histamine was a key mediator, a study, in which plasma histamine concentrations were measured found that the concentrations in healthy dogs and dogs with AD are similar. Cutaneous histamine concentrations were shown to be greater in dogs with AD than in clinically normal dogs, but they did not correlate with the plasma histamine concentrations. In another study, dogs injected ID with histamine did not demonstrate an increase in pruritic behavior. The results of both of these studies further support the concept that AD should not be simplified as a type I hypersensitivity.

**CYTOKINES, LYMPHOCYTES, AND IMMUNOLOGIC ABNORMALITIES**

It became evident that T lymphocytes play a critical role in canine AD and that an imbalance in the T-cell populations characterizes different stages of the disease (eg, Th2 predominance in the acute phase vs Th1 in the chronic phase). Stimulation of peripheral blood mononuclear cells by whole *Dermatophagoides farinae* antigens, or purified major allergens from the mite, resulted in an antigen-specific response in atopic dogs, compared with clinically normal dogs. Great importance was therefore placed on studies focusing on cytokine imbalances, and although dogs did not completely mirror the findings in human medicine, it was demonstrated that a polarization of the T-cell cytokine response may also exist in atopic dogs, as determined by measurement of cytokine transcripts. These studies showed that atopic dogs have a Th2-dominated cytokine response in nonlesional atopic skin in which IL-4 is overexpressed. Interleukin-4 is known to be a major regulatory factor in the production of IgE. Atopic dogs also have low mRNA expression of the immunosuppressive cytokine transforming growth factor-β, compared with clinically normal dogs. This latter finding, if substantiated, provides a possible explanation for the lack of tolerance to environmental allergens in dogs with AD. In lesional atopic skin, a mixed cytokine transcription profile is seen in which Th1 cytokines such as IL-2, interferon-γ, and tumor necrosis factor-α as well as IL-4 are overexpressed. This suggests that in chronic skin lesions, a mixed Th1-Th2 response is seen, possibly associated with self-trauma or secondary infection. Once an experimental model for canine AD was established, it was possible to evaluate the kinetics of cytokine transcription involved in the allergen-induced lesions via the atopy patch test model. Results of those studies show that the cytokine transcription pattern changes over time with a biphasic response. More specifically, Th2 cytokines may play a more important role in the acute phase (12 to 24 hours after allergen exposure), whereas Th1 cytokines may be more relevant in the chronic phase (48 to 96 hours after allergen exposure). The dynamic nature of the process may help explain the variability in the results reported in studies involving clinical patients in which it is unknown when specific lesions actually started within the dynamic process. Further research to demonstrate the presence of biologically active cytokines is required, however, before the role of cytokines in canine AD can be actively determined.
effects of treatment with essential fatty acids. One of the proposed mechanisms of action of such treatment is the competition of the essential fatty acids with arachidonic acid for cyclooxygenase and lipoxigenase enzymes, resulting in a modification of LT synthesis and reduction of highly proinflammatory products (e.g., LTB₄).

Controversies still exist on the importance of LT in canine AD. Ultimately, it appears that AD cannot be explained with a single mediator, antibody, or cytokine, but it is the result of a multitude of mediators that all contribute to the final inflammatory process.

**Abnormalities in the barrier function of the skin**

Recent research has rediscovered the importance of an impaired skin barrier in humans with AD. Although the role of allergens in the pathogenesis of AD in humans has been known for a long time, the combination of impaired skin barrier with allergen exposure has helped explain the development of an IgE response to the offending allergens. It is indeed known that if a substance is repetitively applied on a disrupted skin, an IgE response to such substance will ensue. Therefore, the IgE, allergens, and skin barrier impairment are all different and important aspects of the same clinical syndrome.

Evidence of skin barrier dysfunction in dogs with AD has also been rapidly building. After an initial pilot study documented irregularities (e.g., lipid lamellae thinning and discontinuity) in the ultrastructure of the upper layers of the epidermis of atopic dogs with naturally occurring disease, several more recent studies have described both ultrastructural as well as functional changes of the skin barrier function. These studies were performed on dogs with naturally occurring AD as well as dogs with experimentally induced AD. These impairments are both ultrastructural and functional as measured by increased transepidermal water loss.

More specifically, ultrastructural changes in the stratum corneum were reported to be already present in nonlesional atopic skin and were aggravated after allergen exposure and development of skin lesions. These included widening of the intercellular spaces as well as retention of lamellar bodies in the corneocytes and irregularities and fragmentation of lipid lamellae. These changes are strikingly similar to the ones reported in humans with AD, in which a disturbed extrusion of lamellar bodies is thought to be responsible for the trapping of these organelles inside the corneocytes.

In humans with AD, the ultrastructural changes are accompanied by a decrease in epidermal ceramides. This decrease has been explained by increased degradation due to multiple enzymatic alterations. Results from previous studies indicate that atopic dogs also have decreased ceramides, but it is presently unknown whether this is due to an enzymatic alteration, a disturbance in organelle extrusion, or both. Also, it is presently unknown whether the ultrastructural changes noted in canine nonlesional skin are due to a primary defect or are secondary to subclinical inflammation. What is known is that the skin has increased transepidermal water loss and is therefore more permeable, particularly in young dogs. In humans, it is proposed that the increased permeability of atopic skin combined with genetic predilection toward a Th2 response plays an important role in increasing the allergen penetration and the risk for allergic sensitization. Because the percutaneous route of allergen exposure appears to be important in dogs, it is possible that a similar scenario as that proposed for the pathogenesis of AD in humans may also apply to dogs.

**Routes of allergen presentation and processing**

In early reports of dogs with AD, the disease was termed allergic inhalant dermatitis. Although respiratory signs can be seen in some dogs with pruritic (allergic) skin disease, this is uncommonly reported. The inhalant route of allergen exposure has been shown to contribute to AD in dogs but does not appear to be the main route of allergen exposure. Recent studies have provided evidence that the most important route of allergen presentation in dogs with AD occurs percutaneously. This might explain the distribution of the clinical lesions seen in affected dogs. In general, lesions and pruritus are worse on the limbs, particularly the caudal aspect of the carpus and tarsus, ventral aspect of the abdomen, perioral and periorcular skin, and pinnae.

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**Role of secondary infections and skin barrier**

The challenge of managing AD in dogs also lies in the frequent secondary infections that aggravate the clinical signs. Atopic skin is more prone to be colonized by Staphylococcus spp, and this may occur because of a variety of factors, ranging from decreased antimicrobial peptides to increased adherence due to the overexpression of Th2 cytokines. Colonization can result in an increase in IL-4 and IL-13 from cutaneous T cells. This increase in IL-4 can induce the production of fibronectin, which can contribute to the adherence of Staphylococcus spp to keratinocytes. Colonization by Staphylococcus spp further damages atopic skin as bacteria produce ceramidases and proteases, which additionally decrease ceramides in the stratum corneum and create a self-perpetuating cycle of disruption of skin barrier and inflammation.
Role of mast cells and other effector cells

Numerous inflammatory cells are thought to play a role in the pathogenesis of canine AD, although in the past, mast cells were considered the most important. But evidence for this assumption is lacking, and it is likely that a complex interplay exists between wide varieties of cell types. The cells that play a pivotal role in the pathogenesis of canine AD are Langerhans cells and dermal dendritic cells, which are responsible for antigen processing and presentation, B lymphocytes that are responsible for reaginic antibody production; allergen-specific helper T lymphocytes that are responsible for cytokine production, leading to activation of B cells and other inflammatory cells, and mast cells that produce inflammatory mediators, leading to inflammation. In terms of cell numbers seen by histologic examination of sections of lesional atopic skin, mononuclear cells would appear to have the predominant role, but it is not clear whether this high cell density is correlated to pathogenicity.

Hypothesized pathogenesis of canine AD—Taking into account a recent study on the pathogenesis of canine AD, it is possible to postulate a pathway that is likely to occur in the atopic skin of dogs. Atopic dogs may be genetically predisposed to having defective epidermal barrier function and polarization of lymphocytes toward the Th2 subset. A deficiency of transforming growth factor-β in the skin could lead to a lack of tolerance toward environmental allergens (especially the high–molecular weight D. farinae allergen Der f 15), which would penetrate the epidermis and be intercepted by Langerhans cells. The Langerhans cells would process the antigen and present it to T lymphocytes in the draining lymph node and drive Th2 polarization. Overproduction of IL-4 by the lymphocytes would lead to class switching by B cells and production of allergen-specific IgE, which would bind to cutaneous mast cells. Degranulation of mast cells following exposure to allergen as well as homing of lymphocytes to the skin would lead to cutaneous inflammation. Additional cytokines released by the T cells would lead to pruritus and self-trauma, which, in conjunction with the secondary infections, could lead to development of Th1-driven inflammation in the chronic phase. Hence, successful management of AD in dogs is likely to require reversal or control of the above pathways (Figure 2).

Where We Are Going

Improvements in the treatment and management of dogs with AD can be made only when there is a better understanding of the disease process. This includes more research into the alterations involved in barrier dysfunction, the cell types and tissues contributing to this complex disease, and the signaling pathways mediating pruritus and inflammation.

Skin barrier—The ongoing debate regarding the pathogenesis of AD is whether it is caused by a primary immune defect, leading to skin inflammation (inside-outside hypothesis), or whether the inflammation of AD is a result of a primary defect in the skin barrier (outside-inside hypothesis). The key to understanding this complex disease may lie in the fact that different aspects may be important at different stages of development and that overlap exists. For example, some individuals may start with a primary defect of skin barrier, whereas others may not. But ultimately, once allergic sensitization has occurred and inflammation has developed, decreased skin barrier function occurs, leading to a self-perpetuating cycle of sensitization, inflammation, and skin damage.

Future study in the area of barrier dysfunction should include identifying genetic defects in dogs with AD that might lead to the formation of a defective skin barrier. A recent study in atopic dogs that involved the use of immunofluorescent microscopy revealed that 15 of 18 dogs had abnormal expression of the filaggrin protein, a protein produced by skin cells that degrades into amino acids during skin cell maturation and that is essential for maintaining moisture in the outer layers of intact skin. Four of 18 dogs had a filaggrin-staining pattern consistent with a loss of function mutation in the filaggrin gene. In affected humans, several loss-of-function mutations in the same filaggrin gene have been identified and are considered a major predisposing factor for AD. The consequence of a loss of function mutation in filaggrin, for example, is the formation of dry, flaky skin that is more permeable to allergens, pathogens, and chemical irritants. Skin permeability to foreign substances likely engages the immune system and primes the body to react to antigens that it would not normally encounter by this route.

If the importance of skin barrier dysfunction proves to be primary in dogs as it is in humans, it may greatly change the way veterinarians approach this disease. For one, more preventative treatments aimed at restoration of the skin barrier to minimize allergen penetration and sensitization would need to be considered. It is already known that topical application of ceramide-rich preparations can help improve the skin ultrastructure in atopic dogs. What is not known is whether any improvement in the ultrastructure of the skin is paralleled by clinical improvement, as demonstrated in human medicine, where the clinical efficacy of ceramides applied topically is impressive. Future clinical studies in veterinary medicine should focus on evaluation of the clinical efficacy of topical treatments aimed at improving barrier function and the correlation of the improvement of skin barrier with severity of clinical signs in AD.

Keratinocyte function in skin barrier

Keratinocytes are the primary cells that lead to skin barrier formation through the process of epidermal differentiation and migration. As keratinocytes mature, they accumulate keratin and lipids such as cholesterol, free fatty acids, and ceramides. As they migrate from the stratum granulosum to the stratum corneum, the cells rupture and thus form a dense protein and lipid-rich barrier capable of preventing the entry of harmful substances from the environment.

Defects in keratinocyte function have been identified in humans with AD and could contribute to skin barrier dysfunction. For example, abnormal keratinocyte proliferation and differentiation have been found in non-
Lesional and lesional skin of atopic humans and can be associated with reduced expression of certain keratins and changes in expression of cornified envelope proteins such as involucrin and loricrin. These findings have led some groups to search for ways to induce human skin cells to produce more proteins involved in skin barrier formation, discover topical treatments that may allow normal lipid production and secretion, or find ways to reduce proliferation of human keratinocytes in lesional skin. Such approaches to treatment may also be useful for the treatment of affected dogs if barrier dysfunction becomes a primary driver in canine AD.

Figure 2—Depiction of the currently proposed pathophysiologic mechanism of canine AD based on research findings in dogs and humans with AD. The disease process starts with percutaneous exposure and absorption of allergens through an epidermis that may have a defective barrier function. Left panel—The process of sensitization in canine AD. The naïve Langerhans cell captures and internalizes allergens. Allergens are then processed, packaged in major histocompatibility complex molecules on the Langerhans cell surface, and presented to naïve T helper cells (Th0) cells in the draining lymph node. Specific cues from the microenvironment enable dendritic cells to activate T helper cells and polarize them toward a Th2 phenotype, where they produce cytokines such as IL-4 and IL-13. These cytokines can stimulate B cells to become plasma cells that begin producing allergen-specific IgE. Activated Th2 cells migrate to the skin with the help of chemokines produced by various cells in the skin, such as thymus and activation-regulated chemokine. Allergen-specific IgEs also enter into the circulation and other tissues and bind to cells expressing high- and low-affinity Fce receptors on their cell surface. Right panel—Progression of canine AD in terms of neurologic and immune components of disease. Upon reexposure to the same allergen, the epidermal Langerhans cell with cell surface–bound allergen-specific IgE efficiently binds the allergen and migrates to the dermis. These Langerhans cells then present the allergen to T helper lymphocytes and continue to polarize them toward a Th2 phenotype. Additional Th2 cytokines such as IL-13 can be released and activate the sensory neuron to induce pruritus. Allergens can also cross-link allergen-specific IgE bound on the cell surface of dermal mast cells and stimulate the release of preformed inflammatory mediators such as histamine, serotonin, and substance P along with cytokines such as eosinophil chemotactic factor. Skin injury by scratching, microbial toxins from Staphylococcus sp and Malassezia sp, or environmental allergens activate keratinocytes and other innate immune cells to release proinflammatory cytokines (eg, IL-12) and chemokines that can polarize T helper cells toward a Th1 phenotype, where they produce cytokines such as interferon (IFN)-γ. In turn, IFN-γ promotes monocyte-macrophage cell activation. Activated keratinocytes, monocytes, and mast cells produce additional proinflammatory cytokines such as tumor necrosis factor (TNF)-α, upregulating the expression of P-selectin and E-selectin, on endothelial cells, thus recruiting more leukocytes from the blood. The epidermis thickens as does the stratum corneum; the barrier function worsens, allowing increased allergen penetration; and the cycle is perpetuated. TSLP = Thymic stromal lymphopoietin.

Keratinocyte function in innate immunity

Keratinocytes also have a great capacity to respond to environmental insult or invading organisms such as bacteria, viruses, and fungi by producing cytokines, chemokines, and antimicrobial peptides. Many of these responses lead to the protection against infection, control of inflammation, promotion of wound healing, and communication with the nervous system. When these processes are dysregulated, however, they can contribute to the pathophysiologic features of AD.

Canine keratinocytes have already been shown to produce pro-inflammatory agents such as granulocyte macrophage colony-stimulating factor, IL-8, and tumor necrosis factor-α, in response to allergens and bacterial components such as Der f1 and lipopolysaccharide, respectively. An additional keratinocyte cytokine of great interest that has been implicated in AD in humans includes thymic stromal lymphopoietin. Thymic stromal lymphopoietin is an IL-7-like cytokine that stimulates dendritic cells to induce naïve T cells to differentiate into Th2-like cells. Thymic stromal lymphopoietin has been shown to be high in lesional skin of humans with AD, and it can stimulate naïve T cells to produce pro-allergic cytokines such as IL-4, IL-5, and IL-13. Genetic variants in thymic stromal lymphopoietin have also been found in humans and are associated with AD.
Keratinocyte production of many antimicrobial peptides can also be altered in AD and may contribute to the abnormal skin barrier against infection. However, results of studies on humans with AD are not clear cut. Two studies\textsuperscript{102,103} have shown that antimicrobial peptide induction (cathelicidin and β-defensins hBD-2 and -3) is greatly decreased in lesional skin of humans with AD and is associated with an increased susceptibility to microbial infections such as \textit{Staphylococcus aureus} and herpes simplex virus. But another study\textsuperscript{104} revealed enhanced expression of antimicrobial peptides (RNase 7, psoriasin, and hBD-2 and hBD-3) in lesional skin of humans with AD and no correlation with \textit{S. aureus} colonization. There has been 1 study\textsuperscript{105} in dogs with AD in which the cutaneous content of antimicrobial peptides was evaluated. In that study,\textsuperscript{105} it was found that the \( \beta \)-defensin cBD1 was significantly increased in lesional skin, whereas cBD103 was downregulated. Clearly, the role of antimicrobial peptides regarding secondary skin infections in AD needs further evaluation.

The skin of atopic dogs is often colonized with bacteria such as \textit{Staphylococcus pseudintermedius} and \textit{Malassezia pachydermatis},\textsuperscript{106} so treatments that could regulate the cutaneous concentration of antimicrobial peptides and reduce cutaneous infections would be valuable. Some benefits of antimicrobial peptides acting as antimicrobial agents include the following: they kill a broad spectrum of microbes, and microbe resistance is essentially absent because their mechanism of action is via physical interaction with microbial membranes through their cationic charge and hydrophobic amino acids.\textsuperscript{107} Future research in this area is clearly needed, and results of clinical trials in humans with AD to evaluate the efficacy of vitamin D3, which regulates cathelicidin expression in keratinocytes, will also be of great interest.\textsuperscript{108}

Inflammation and immune dysfunction—Factors other than barrier dysfunction play a critical role in the pathophysiologic mechanisms of AD, such as dysregulation of immune cell function and inflammation. A large amount of research has focused on the immune system's hypersensitization to environmental allergens, allergen-specific IgE production, mast cell degranulation, and a biphasic T-cell response within the skin (Figure 2). Updated information on the role of proinflammatory mediators in AD and the identification of novel cellular sources of these agents has been generated for the human disease condition and may provide additional areas of research to better understand the pathophysiologic features of canine AD.

Dendritic cells such as the Langerhans cells are present in the epidermis and dermis of the skin and are thought to be critical players in the pathogenesis of canine AD. Dendritic cells function as antigen-presenting cells and present antigen to T lymphocytes, initiating the primary and secondary adaptive immune responses. After initial allergen sensitization, Langerhans cells in the epidermis and dermis have been shown to bind allergen-specific IgE and can continue to respond efficiently to allergens, driving the Th2-like cytokine response.\textsuperscript{17,12} Recently, human dermal dendritic cells have been shown to produce IL-25, a member of the IL-17 cytokine family. This cytokine induces the production of Th2-associated cytokines and reduces the production of filaggrin by keratinocytes. Furthermore, high concentrations have been detected in the skin from humans with AD.\textsuperscript{109–111} These studies\textsuperscript{99–111} suggested that dendritic cells may have additional roles in AD beyond antigen presentation such as inducers of the Th2 response in the skin and contributors to barrier dysfunction.

Macrophages are a major cell type detected in the cellular infiltrate in chronic lesions of AD in humans.\textsuperscript{112} They are also commonly observed in skin biopsy specimens from dogs with AD.\textsuperscript{113} These cells play a key role in the initiation, propagation, and resolution of inflammation and have great capacity to produce proinflammatory mediators such as IL-1, IL-18, IL-6, and tumor necrosis factor-α as well as cytokines that aid in the resolution of inflammation (eg, transforming growth factor-β).\textsuperscript{114} This cell type has been implicated in a variety of chronic inflammatory diseases in humans and is likely to be a key player in the development of canine AD.

T-helper cells play a key role in the pathogenesis of AD. Biphospho T-helper cell responses are seen in humans with AD in which Th2 cytokine mRNA production occurs acutely (eg, IL-4, IL-5, and IL-13) and then Th1 (eg, interferon-γ) along with Th2 cytokine message production occurs in the more chronic form of the disease.\textsuperscript{115} These T-cell cytokines can cause detrimental changes in the skin, such as decreased expression of barrier proteins, antimicrobial products, and adherence factors as well as decreased viability of keratinocytes and increased susceptibility to cutaneous infections.\textsuperscript{115,116}

Newer Th1- and Th2-cytokines have been identified and have been the focus of several recent studies.\textsuperscript{117,118} For example, IL-21 is a cytokine produced by Th1 cells in humans and appears to be involved in a variety of inflammatory processes in the skin, such as enhanced proliferation and function of natural killer cells, lymphocytes, and keratinocytes.\textsuperscript{119} Differentiation of B-cells into plasma cells\textsuperscript{21}, differentiation of naïve T cells into Th1-helper type 17 cells\textsuperscript{121,122}; and suppression of regulatory T-cell function.\textsuperscript{124} Recently, studies\textsuperscript{120,125} have shown that IL-21 and its receptor are upregulated in skin lesions of patients with AD, suggesting this cytokine may contribute to the pathophysiologic features of skin disease.

Another recently identified cytokine of interest is IL-31.\textsuperscript{126} This cytokine was found to be produced by activated Th2 lymphocytes and expressed in cutaneous lymphocyte antigen-positive skin homing T cells in human patients with AD.\textsuperscript{125,127} Interleukin-31 binds to a heterodimeric receptor consisting of the IL-31 receptor A and the oncostatin-M receptor β.\textsuperscript{128,129} These receptors are found on a variety of cells such as keratinocytes, macrophages, and eosinophils and participate in regulating immune responses in these cell types.\textsuperscript{130,131} A link between IL-31 and AD has been shown by studying the phenotype of IL-31 transgenic mice. When IL-31 is overexpressed in mice, they have severe pruritus, alopecia, and skin lesions,\textsuperscript{132} and the pruritic effects of IL-31 can be ameliorated by an anti–IL-31 antibody.\textsuperscript{132} Other groups have evaluated the role of IL-31 in humans with AD. For example, Sonkoly et al\textsuperscript{133} found high concentrations of IL-31 mRNA in the skin of humans with AD. Whether IL-31 causes scratching behavior and dermatitis in dogs, as seen in mice, still needs to
be determined. One study,\textsuperscript{135} in which the nucleotide sequence of canine IL-31 was determined, did not find high mRNA concentrations in the skin of atopic dogs. Recently, the role of other types of T-helper cells in AD such as T-helper type 17 cells has been highlighted.\textsuperscript{136} T-helper type 17 cells not only appear to play a role in protective immunity against extracellular pathogens but can also be potent inducers of tissue inflammation. These CD4\textsuperscript{+} T cells have been shown to produce cytokines such as IL-17, IL-22, and IL-25.\textsuperscript{137} Interleukin-17 appears to be expressed in acute lesions in AD but is largely absent in chronic AD lesions,\textsuperscript{138,139} allowing some to hypothesize its reduction may contribute to persistent infections in the skin of AD patients.\textsuperscript{136} Interleukin-22, however, is a cytokine that is upregulated in chronic AD skin lesions and has been shown to downregulate genes involved in terminal differentiation of the skin, which could lead to epidermal hyperplasia.\textsuperscript{138} Interleukin-23 plays an important role in driving Th2 polarization and has been shown to induce the production of IL-4, IL-5, and IL-13.\textsuperscript{109,110} Abnormal regulatory T-cell responses may also play a role in AD. Regulatory T-cells are a specialized population of cells that maintain immune homeostasis or peripheral tolerance\textsuperscript{140,141} and have been shown to suppress allergen-specific T-cell activation.\textsuperscript{141} There have been studies\textsuperscript{140,142} evaluating the presence of regulatory T-cells in lesional skin of humans with AD, and results are mixed.

**Neuroimmune interactions in the skin**—There is increasing evidence suggesting a synergistic interaction between the nervous system and the immune system within the skin.\textsuperscript{143,144} Resident immune cells such as mast cells, Langerhans cells, and transient immune cells present during inflammation (eg, granulocytes and T lymphocytes) are intimately associated with nerve fibers. When such immune cells are activated, they can release substances such as neuropeptides (eg, histamine and substance P), cytokines (eg, IL-31), and neurotrophins (eg, nerve growth factor) that can bind directly to receptors on sensory nerves to cause activation, sensitization, and sprouting of nerve cells. Similarly, activated nerves can release neuropeptides (eg, substance P and calcitonin gene–related protein) and neurotrophins (eg, nerve growth factor) that can modulate immune cells and their responses during inflammation. As a result of the complex innervation of the skin with sensory nerve fibers, immune cells and sensory nerve fibers clearly communicate with one another and regulate each other’s activity. They also likely participate synergistically in the pathogenesis of skin diseases.

In the human with AD, such mediators have been found to be upregulated. For example, IL-31, the Th2 cytokine expressed in cutaneous lymphocyte antigen–positive skin homing T cells, is preferentially found in higher amounts in pruritic versus nonpruritic skin conditions.\textsuperscript{143,144} Interestingly, the receptors to this cytokine, the IL-31 receptor A and the oncostatin-M receptor β, have most recently been found on sensory C-fibers and in the dorsal root ganglia in rodents where they likely contribute to the transduction of pruritus signals.\textsuperscript{146,147}

An important neurotrophin in the skin that can be dysregulated in AD is nerve growth factor. This molecule is produced by a variety of cells in the skin including keratinocytes, mast cells, eosinophils, and lymphocytes. It binds and exerts its effects through the low-affinity pan-neurotrophin receptor p75\textsuperscript{NTR} and the high-affinity neurotrophin receptor of the tyrosine kinase family, and these receptors are present on keratinocytes, immune cells, and neurons. Nerve growth factor has a variety of functions that include tissue remodeling, immune cell activation, and neuronal growth.\textsuperscript{147} Enhanced expression and release of nerve growth factor from skin cells have been found in humans with AD,\textsuperscript{148} and nerve growth factor serum concentrations correlate with disease severity.\textsuperscript{147,149,150} Suggesting neurotrophic factors such as nerve growth factor can have a pathogenic role in skin diseases when dysregulated.

Neuropeptides can also contribute to pathogenic conditions in the skin. For example, the neuropeptide substance P can be localized in primary cutaneous sensory nerves and released by nerve endings in the periphery after activation. Substance P can then bind neurokinin-1 receptors present on a variety of immune cells such as neutrophils, lymphocytes, macrophages, lymphocytes, and mast cells, leading to their activation.\textsuperscript{151} Neurokinin-1 receptors have also been found on neurons within the dorsal horn, and recently, these neurons have been shown to mediate scratching behavior in mice.\textsuperscript{152} Substance P concentration has been found to be high in the plasma of atopic humans,\textsuperscript{149} suggesting that neuropeptides may also contribute to the pathogenesis of allergic skin disease.

**Pruritus**—Pruritus is the hallmark of canine AD. The ability to develop effective treatments for pruritus has been hampered by the poor knowledge of the underlying pathways and mechanisms. In mice, rats, and nonhuman primates, it has been shown that the itch and pain sensations are transmitted by distinct neurons.\textsuperscript{153,154} The itch signals are detected through relevant itch receptors present on cutaneous itch-selective sensory nerves residing in the epidermis and dermis. The signals then travel along unmyelinated C nerve fibers and are received by the dorsal root ganglia and the lamina I region within the dorsal horn of the spinal cord. The itch signal finally reaches the brain through spinothalamic tract neurons.\textsuperscript{153,154}

Nerve ablation techniques in rodents have identified some of the characteristics of itch-selective neurons in the spinal cord. For example, 1 study\textsuperscript{152} suggested that NK-1 receptor–expressing dorsal horn neurons play a key role in scratching behavior and a second study\textsuperscript{155} suggested that gastrin-releasing peptide receptor–expressing neurons in the lamina I of the dorsal horn selectively modulated a variety of itch responses but do not affect pain responses in rodents. If these characteristics of itch-selective neurons in rodents translate similarly into dogs, then a variety of new treatment approaches to pruritus could be explored.

One of the most commonly used treatments for allergic skin disease in humans is antihistamines, which antagonize the histamine H1 and H2 receptors present in a variety of tissues including periph-
eral neurons, blood vessels, and smooth muscle cells and works by reducing pruritus, pain, and vascular permeability. Although antihistamines are commonly recommended by veterinary dermatologists for the treatment of pruritus associated with AD in dog, their efficacy is unclear.156

Newer agents with novel mechanisms have been shown to be active in rodents with experimentally induced pruritus. These agents include histamine H4 receptor, μ opioid receptor antagonists, κ-opioid receptor agonists, protease-activated receptor 2 antagonists, serotonin antagonists, α2δ ligands, cannabinoid receptor agonists, and neurokinin-1 receptor antagonists.143,155 Some of these approaches are currently being investigated in controlled human clinical studies to examine their antipruritic effects.

Intracellular mechanisms—A great complexity of intracellular signaling mechanisms is used by cytokines, chemokines, neuropeptides, and neurotrophins. Many neuropeptides and chemokines exert their effects via G protein–coupled receptors,158 and many cytokines and neurotrophins will activate receptors that signal through a variety of pathways including the Janus-activated kinase signal transducer and activator of transcription pathway,159 mitogen-activated protein kinase pathway,160 phosphatidylinositol 3-kinase pathway,161 nuclear factor kappa B pathway,162 or nuclear factor of activated T cells pathways.163 These signaling pathways have been well described, and a variety of small molecule inhibitors have been developed to target many of these pathways or are currently under clinical investigation in diseases such as oncology, transplantation, and autoimmune disorders. One example currently used for the treatment of dogs with AD is cyclosporine. This drug binds to a cytoplasmic protein, cyclophilin. The cyclosporine-cyclophilin complex then inhibits calcineurin, an enzyme with serine-threonine phosphatase activity, preventing the dephosphorylation and activation of nuclear factor of activated T cells. Nuclear factor of activated T cells, if activated by dephosphorylation, would normally translocate to the nucleus and induce the expression of IL-2 in T cells. Therefore, cyclosporine is a signal transduction inhibitor, functioning in part by blocking T-cell activation.164 As the safety and efficacy of other signal transduction inhibitors being evaluated in human diseases become better characterized, some may have value in the treatment of dogs with AD.

Diagnosis of Canine AD

Currently, there is no definitive test to confirm a diagnosis of AD in dogs. Veterinarians therefore spend a substantial amount of time ruling out infections, infestations, and food allergies before intradermal testing and serologic tests are performed in these animals. Serum-based diagnostic or genetic testing that could be used in collaboration with clinical assessments may help veterinarians prescribe the most appropriate treatments for AD patients more quickly. There appears to be a strong link between dog breed and the development of AD, suggesting a genetic link does exist165,166 and diagnostic testing could be useful. A recent study by Wood et al167 evaluated a variety of single nucleotide polymorphisms in 23 candidate genes in 242 dogs with AD and 417 healthy control dogs across several breeds and regions. Initial findings of that study167 suggest there are candidate genes associated with the disease and clearly support further genetic studies with larger numbers of dogs of individual breeds from defined geographic regions to strengthen genetic associations. Understanding the function of genes associated with AD may also lead to serum-based diagnostic testing that could be made available to veterinarians.

Summary

During the 70 years that veterinarians have been diagnosing and treating pruritic allergic skin disease in dogs, much has changed. Atopic dermatitis was originally thought to be a type I hypersensitivity with inhaled allergens as the main cause, and much of the research on the pathogenesis and treatment focused on mast cells and allergen-specific IgE. It is now known that AD is a multifactorial disease that instead has cutaneous dendritic cells, T lymphocytes, a multiplicity of other cells, and an altered barrier function at the center of the disease process. In dogs that may have a genetic predisposition or congenital alteration in epidermal permeability, environmental allergens can penetrate this cutaneous barrier and trigger a complex immunologic reaction. Many different cytokines released by these cells are the signals that drive this process. Cutaneous lesions of inflammation coupled with neuronal mechanisms start the never-ending cycle of pruritus. Among dogs and also over time within an individual dog, the disease process is continuously changing. This adds to the challenges in making an accurate diagnosis and suggests that no single treatment will ever be universally effective.

As research in humans and dogs as well as other animals progresses, the many parts and complex interplay to AD are slowly being uncovered. With this new information, more effective and safer modes of treatment targeting the disease at many points in the cycle are now possible.

References

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