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Canine Atopic Dermatitis: New Targets, New Therapies¹

Douglas J. DeBoer²

*Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin,
Madison, WI 53706*

ABSTRACT Atopic dermatitis is a common allergic skin disease of complex etiopathogenesis in both humans and dogs. Immediate-type hypersensitivity to environmental allergens that arises as a result of environmental and genetic factors is a major part of the pathogenesis in most but not all patients. Alterations in epidermal barrier function, priming of cutaneous antigen-presenting cells with IgE, intrinsic keratinocyte defects, and even development of autoimmunity are also factors that contribute to the primary disease. Secondary factors, especially infections with *Staphylococcus* and yeast organisms, strongly influence the course of this skin disease. The relatively recent understanding of the complexities of atopic dermatitis has resulted in changes in diagnostic and therapeutic strategies for the disease. We now know that the best therapeutic approach is to use combinations of multiple modalities individualized for each patient over the course of his or her lifetime. *J. Nutr.* 134: 2056S–2061S, 2004.

KEY WORDS: • allergy • atopic dermatitis • canine • hypersensitivity

Atopic dermatitis (AD)³ is one of the most common pruritic skin diseases of dogs. During the past 10 years, concepts regarding the pathogenesis of AD have evolved substantially, including mechanisms involved in the primary disease and the role of secondary cofactors. These new findings have profound effects on the present approach to AD diagnosis and treatment. Management of AD now requires that we view the large number of available treatment options as tools, the challenge being to select which combination of tools will provide best long-term control for an individual patient. We must ask which factors are involved in the pathogenesis of the primary disease and how these might be mitigated, but at the same time, we must pay attention to equally important secondary cofactors that may promote, augment, or exacerbate the disease.

It is important to emphasize that most of these concepts were developed and studied on human and/or rodent models. It is far from clear whether the same mechanisms operate in dogs; however, thus far, what has been discovered for dogs has proven remarkably close to established concepts in humans.

The role of IgE-mediated hypersensitivity

Canine AD used to be viewed as a straightforward, IgE-based, immediate-type hypersensitivity reaction to inhaled environmental allergens, as is evident in the former name for this condition in dogs: “allergic inhalant dermatitis” (1). It was hypothesized that an aberrant immune response led to inappropriate allergen-specific IgE production, and the IgE bound to mast cells in the dermis. On subsequent allergen exposure, mast cell degranulation ensued with release of mediators such as histamine, and clinical signs were thus produced. Conventional conservative therapy included antihistamines and antiinflammatory fatty acid supplements. Use of the H1-receptor-antagonist drugs (such as diphenhydramine) to block the effect of histamine as a mediator met with limited success, although the drugs and dosages used were extrapolated from human medicine and may not have been optimal for animal use. Likewise, using fatty acid supplements in an attempt to interfere with the arachidonic acid cascade and production of inflammatory prostaglandins and leukotrienes was minimally beneficial (1). This limited therapeutic success could have been a warning that the pathogenesis of AD is actually much more complex than previously thought, although clearly, IgE-mediated hypersensitivity remains an essential component of the pathogenesis of AD for at least the majority of canine and human patients.

Upon exposure to most foreign antigens, the usual or “normal” humoral immune response results in production of IgG antibody rather than IgE. A major determinant of which antibody class predominates is which one of two subsets of T-helper lymphocytes (designated Th1 and Th2) is dominant. These subsets are characterized by different profiles of cytokine release upon activation (2). Th1 activation, the “normal”

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² To whom correspondence should be addressed. E-mail: deboerd@svm.vetmed.wisc.edu.

³ Abbreviations used: AD, atopic dermatitis; APC, antigen-presenting cell; ASIT, allergen-specific immunotherapy; LPR, late-phase response; Th1 and Th2, T-helper lymphocytes.

response, results in release of cytokines such as $\text{INF-}\gamma$ and IL-2, which act to promote IgG production. If Th2 cells are activated instead, they release IL-4, -5, -13, and other proallergic cytokines, which results in recruitment of eosinophils into the inflammatory site and induces Ig-class switching in lymphocytes to result in production of IgE rather than IgG. The factors that determine whether a Th1 or a Th2 response will predominate are complex but include both genetic and environmental influences.

Studies on humans have demonstrated the predominance of Th2 lymphocytes and their products in early AD lesions (3), but only a few of these observations have been extended to dogs. The skin of dogs with AD overexpresses IL-4 mRNA and underexpresses transforming growth factor- β compared with healthy dog skin, which indicates a Th2-biased response (4).

In the last 20 years, many aspects of the structure and function of IgE and its receptors have been elucidated along with mechanisms for regulation of these pivotal molecules. In humans (and perhaps in dogs), IgE may now be thought of as a "family of molecules" with extremely complex regulatory mechanisms for control of synthesis and activity (5,6). To date, no specific functionality or pathogenetic roles have been uncovered for different isoforms of IgE.

Is AD *always* IgE mediated? Importantly, physician allergists now recognize two different forms of AD in humans. In the "extrinsic" form, there is a family history of allergy and positive skin and serum allergen-specific IgE tests. In the "intrinsic" form, patients have identical clinical symptoms and a family history of allergy yet do not show positive results with the same "allergy tests" (7). The latter patients represent ~30% of all human AD incidence and raise the question as to whether AD is always purely IgE mediated. It has become abundantly clear that there is more to allergy than just IgE; this fact has also caused us to reconsider the role of IgE-based allergy tests in the overall diagnosis of AD. Perhaps we should not expect that all dogs with a clinical diagnosis of AD will show positive results on an allergy test—other factors seemingly unrelated to IgE-mediated hypersensitivity may explain the clinical signs.

For those AD patients in whom allergen-specific IgE is present in sufficient quantities to permit *in vitro* detection, screening tests for serum IgE may permit more cost-effective diagnostic evaluation of allergy. These assays test for the presence of serum IgE directed against a mixture of common allergens. In theory, if a serum sample contains sufficient allergen-specific IgE to be detectable in an immunoassay using a mixture of allergens, this IgE may then be detectable in an individual-antigen immunoassay using the same sample. Conversely, if no allergen-specific IgE is detected in a mixed-allergen screening test, it is more likely that the individual-allergen test will be negative. Thus if the initial inexpensive screening test shows a positive result, the client has some incentive to bear the cost of the full allergy-testing panel with minimum chance that the full panel will produce negative results. Published studies on such screening tests are lacking, however, so the benefits of their use remain theoretical.

Beyond immediate-type hypersensitivity

If IgE-mediated hypersensitivity represents only one component of a more complex pathogenesis for AD, what other factors have emerged as important potential contributors?

Epidermal barrier function. It is now well established that allergens can be absorbed percutaneously instead of, or in addition to, via inhalation. In some humans, alterations in lipid and ceramide compositions were identified in the stratum corneum, which is the uppermost layer of the epidermis that is

critical to epidermal barrier function (8). Decreased barrier function of the skin often is evaluated experimentally by measurement of transepidermal water loss. In many human AD patients, transepidermal water loss is higher, which implies decreased barrier function and leads to the possibility that allergens and irritants can more easily penetrate the skin (9). This concept has not been extended to dogs, however; preliminary and empirical ultrastructural (i.e., electron microscopic) examination of the stratum corneum of atopic dogs revealed differences between normal skin and skin of AD patients (10). In normal dog skin, the keratinocytes of the stratum corneum are arranged in regular, overlapping layers, and intervening lipid material fills the gaps between cells in a classical "bricks and mortar" arrangement. In contrast, the stratum corneum of dogs with AD shows more limited and discontinuous "mortar" between the cells.

If an allergen can penetrate the stratum corneum, is it then capable of eliciting a hypersensitivity reaction? In dogs and humans, the answer is clearly yes. Direct evidence comes from the so-called atopy patch-test model, in which concentrated allergen preparations are applied to skin under an occlusive patch for periods of 24–48 h or more. In dogs sensitive to house-dust mites, application of dust-mite allergen under the patch leads to skin lesions that histologically resemble those of AD (11). Moreover, lesions consistent with AD can be induced merely by exposure of a sensitized dog to an allergen that has been painted onto the kennel surface on which the dog sleeps (12). Thus in an individual who is already genetically susceptible to developing IgE-mediated hypersensitivity, reductions in epidermal barrier function that allow for allergens to enter the skin may contribute to the pathogenesis of AD.

The clinical implications of reduced epidermal barrier function also include those related to treatment. Limiting cutaneous contact with allergens may be helpful to these patients. This may include avoiding contact with the relevant allergen to the extent possible, and also, more frequent rinsing or washing of exposed areas to remove allergens before they can penetrate the skin. Restoring or maintaining epidermal barrier function may become an important part of therapy for AD. The latter could in theory be accomplished through topical applications that in some way restore the limited permeability of the stratum corneum. Preliminary attempts at topical modification of barrier function have met with some success and even clinical improvement in human patients (13,14). Alternatively, methods may be discovered to use dietary means or nutritional supplementation to yield improvement in barrier function.

The late-phase response. We now realize that mast cells are very rich sources of many inflammatory mediators besides histamine. When mast cells are activated, they release a plethora of proinflammatory substances through both granule release and *de novo* secretion through cell membranes. Such mediators include histamine, heparin, leukotrienes, prostaglandins, bradykinin and related molecules, tryptase and chymase, and many others. Importantly, activated mast cells are a rich source of cytokine release. Cytokines (including IL-4) recruit inflammatory cells to the local area beginning 6–12 h after mast cell activation, and the inflammatory cell infiltrate then persists for several days afterward (15). This so-called late-phase response (LPR) is part of the inflammatory cascade that keeps the skin inflamed long past the initial mast cell degranulation and contributes to more chronic inflammatory changes in skin of AD patients. Even just considering the number of mediators released from mast cells and the complexity introduced by the LPR, it is little surprise that mere H1-receptor blockade through administration of antihistamine drugs often results in minimal clinical improvement.

Cutaneous antigen-presenting cells. IgE is present on the surface of mast cells, but importantly, it is now widely recognized in humans and dogs that IgE also has an important role and presence on the surface of cutaneous antigen-presenting cells (APCs). APCs, which are also called dendritic or Langerhans cells, are cells of monocyte-macrophage lineage that function to capture and process foreign antigens and present them to the immune system, thereby initiating an immunologic response. The cells migrate among the epidermal keratinocytes, and upon encountering a foreign antigen, take the antigen in, process it by “digesting” it into smaller pieces, transfer these epitopes to the cell surface, migrate to the local lymph node, and “present” the allergen to the immune system by direct contact with lymphocytes. In part, the nature of the immunologic response that results is related to the type of receptors present on the APC. APCs that express IgE on their surface serve to focus the immune response toward IgE production and immediate-type hypersensitivity. In both atopic humans and dogs and in both lesional and nonlesional skin of these AD patients, there exists a greater number of IgE-bearing APCs and greater expression of the IgE receptor on their surfaces; additionally, the prevalence of such cells correlates well with serum IgE levels (16,17).

The role of keratinocytes. The cells of the epidermis itself (keratinocytes) are now recognized as rich sources of substances that may augment the inflammatory response. When keratinocytes become activated due to trauma, ultraviolet light, irritants, or other causes of skin inflammation, they release >30 different cytokines and related molecules (18). These substances not only recruit inflammatory cells into the skin but also stimulate APC activity and migration.

Activated keratinocytes also release antimicrobial peptides (β -defensins and cathelicidins), which are an important part of the skin’s ability to resist infection. It is now documented that skin cells of human AD patients may have reduced defensin production, and thus may allow colonization of and infection by bacteria (such as staphylococci) much more readily (19). Although this observation has not yet been extended to animals, the parallels between human and canine skin with regard to staphylococcal infection and allergy are striking.

Enhanced secretion of granulocyte-macrophage colony-stimulating factor, IL-1, and tumor necrosis factor- α has been demonstrated from keratinocytes in human AD patients, which suggests that dysregulated signal transduction may occur in these cells (20). Thus part of the pathogenesis of AD may be intrinsic defects in the epidermal cells themselves.

Genetics and environment

AD is a genetically complex disease that develops with gene-gene and gene-environment interactions. Extensive epidemiologic evidence in humans demonstrates the genetic basis of this disease, and this evidence was supplemented recently by abundant molecular genetic studies that show strong association between the atopic phenotype and several different chromosomal regions and specific genes (7,21–26). No one genetic factor or locus explains AD in all patients, however, and it also appears that the proper genetic background is necessary but not sufficient to result in expression of AD. Environmental influences clearly affect whether a human with the genetic background for AD will become clinically atopic. Numerous studies document the higher prevalence of allergic diseases in regions of the world with higher standards of routine healthcare and hygiene. The “hygiene hypothesis” holds that greater exposure to infectious organisms through more limited availability of healthcare and exposure to less-hygienic

conditions tends to promote a Th1 bias to the immune system and a lower prevalence of Th2-biased hypersensitivity disease (27,28). Beyond these findings, candidate gene and genetic linkage studies reveal multiple genes and coding regions on human chromosomes with strong associations to the atopic phenotype (Table 1) (21–25).

A role for secondary cofactors

Staphylococcal infection. We have always recognized that cutaneous infections, particularly staphylococcal infections, are persistent and recurrent in canine AD patients. These secondary infections are emerging as important cofactors in the pathogenesis of AD. Often, the concept that the dog was allergic to the *Staphylococcus* bacteria was put forth as an important contribution to pruritus, and at one time, even intradermal testing with crude staphylococcal antigens was a common procedure. In theory, if dogs developed IgE against staphylococcal antigens, and the IgE subsequently sensitized mast cells, upon reexposure to the staphylococcal antigen, degranulation and mediator release would occur and be accompanied by pruritus and inflammation. Because histamine can inhibit some functions of canine lymphocytes, there was speculation that inhibition of local immunity could occur and perpetuate the infection. Much experimental work was done on staphylococcal hypersensitivity in humans, and IgE against bacterial antigens was detected in certain human patient subsets. In dogs, it was demonstrated that staphylococcal antigens could penetrate the stratum corneum, and that serum of patients with recurrent skin infections sometimes contained detectable anti-staphylococcal IgE (29). However, cause and effect has not been proven, and despite some efforts, the concept of staphylococcal hypersensitivity remains an unproven theory in dogs; if it does exist, it probably does not represent a major allergic mechanism.

In humans, staphylococcal exotoxins functioning as super-antigens have emerged as important contributors not only to clinical signs but also to induction and maintenance of the allergic response, and these molecules may hold the key to the relationship between staphylococcal infections and AD. Staphylococcal toxins such as staphylococcal enterotoxins A, B, C, D, etc. are most familiar for causing food poisoning and enteric disease. However, these molecules are also profoundly active immunologically. They induce potent, direct, non-specific activation of lymphocytes, resulting in cytokine

TABLE 1

Examples of genetic associations made with phenotypically defined atopic dermatitis in humans

Chromosome region	Corresponding molecule or polymorphism coded for by region ¹
5q31–33	Th2 cytokines including IL-3, -4, -5, -13, and granulocyte-macrophage colony-stimulating factor (21)
11q13	Gain-of-function polymorphism in β -chain for high-affinity mast cell IgE receptor (22)
16q12	Gain-of-function polymorphism in IL-4 receptor α -chain (23)
12q21	Interferon- γ and stem cell factor (24)
17q11	RANTES ² promoter region (25)

¹ Parentheses indicate corresponding literature citations.
² RANTES, regulated upon activation, normal T cell expressed and secreted.

production and amplification of the inflammatory response in skin (30). Moreover, they appear able to directly modulate the immune system toward allergy in human studies; for example, exposure of peripheral blood lymphocytes from human AD patients to staphylococcal toxins results in upregulation of IgE synthesis in cells, which is an effect that does not occur in lymphocytes from healthy individuals (31). Unfortunately, canine studies in this area are only just beginning.

Regardless of the exact nature of this relationship, the clinical implications are profound. Infection alone may account for 50–90% of clinical signs in some people and pets with AD as is evidenced by sometimes-dramatic responses to antibiotic treatment (32). Early evaluation and treatment of staphylococcal infections are crucial in treating AD, and long-term infection control is a critical part of lifelong AD management. Options for longer-term control of staphylococcal infections include topical antimicrobial treatments or intermittent use of oral antibiotics. Staphylococcal bacterin therapy has not yet been evaluated for recurrent infection associated with AD, but it may be of aid to such patients in the future.

Yeast dermatitis. Regarding patient comfort, the role of overgrowth or infection of atopic skin with *Malassezia* yeast has no doubt been underappreciated in the past, and merely recognizing and treating it can make a dramatic difference in some canine patients. The presence of IgE against yeast allergens is well documented in cases of human AD (33). We now know that some dogs with AD show a positive response to yeast allergens upon intradermal testing, and some dogs have yeast-specific IgE in their serum. The recent demonstration during passive-transfer experiments that serum from affected dogs can transfer reactivity to normal dogs is primary evidence that IgE-mediated *Malassezia* hypersensitivity exists in dogs (34). Presently we are uncertain about how best to demonstrate the presence of yeast hypersensitivity: neither intradermal testing nor serum-based IgE testing has proven definitive, so clinical criteria are still paramount in diagnosing this condition. Moist or greasy skin with severe pruritus, especially pruritus that is not responsive to corticosteroids, should always prompt the clinician to check for yeast by cytology. Commonly affected areas include the interdigital spaces, axillae, inguinal region, and ventral neck, and skin in these areas may be prominently lichenified and hyperpigmented. The number of yeast found on cytology appears to be unrelated to how severe the clinical signs are; rather, it may be the degree of hypersensitivity that is important. Thus the finding of even one yeast organism on skin cytology of an animal with compatible clinical signs is justification for a treatment trial with oral ketoconazole. Some AD patients have a 50–90% reduction in clinical signs just from treatment of their yeast component. For these patients, long-term treatment with topical antifungal products may be useful.

The issue of chronicity

Several lines of evidence in humans suggest that the character of the cutaneous inflammatory response in AD may change over time. In studies using the atopy patch test, there is a clear predominance of Th2 lymphocytes infiltrating the area early in the course of the lesion; however, over time, with severe, chronic lesions, the pattern changes to a Th1 predominance (35). This suggests a fundamental change in the nature of the inflammatory response in early vs. chronic disease. The clinical implications of this finding are important: early AD and chronic AD may in essence be two different diseases with different responses to treatments and differing prognoses.

Second, self trauma from pruritus exposes structural components of skin in ways that are detrimental to the host.

Fibronectin and other molecules are exposed, which serve as sites for adherence of *Staphylococcus* bacteria to the skin and thus facilitate skin colonization by this organism. Exposure of “self” antigens in the milieu of an active inflammatory response can lead to generation of antibodies against these components; autoantibodies against both epidermal and dermal components have been reported in human patients with AD (36). The antigens are exposed in the vicinity of a great number of IgE-loaded antigen-presenting cells. These may then evoke an IgE autoantibody response, which in essence is the development of a hypersensitivity response against the self. Thus the inflammatory response may be initiated by environmental allergens, but with chronicity, it may be maintained by autoallergens. In this case, even complete removal of the environmental allergens may not create remission of chronic disease.

From a clinical standpoint, adverse events occurring with chronicity argue strongly for early intervention and treatment of AD, when the disease may be more manageable and the potentially irreversible chronic changes have not yet occurred.

Implications: what works in the clinic, and what doesn't?

Physician and veterinary dermatologists and allergists use a variety of therapeutic modalities to provide lifelong management for their patients. Historically, AD is a disease that is most effectively treated using a combination of different therapies. Only now are we beginning to appreciate from a pathogenetic standpoint why this is true.

Immunologic intervention. Allergen-specific immunotherapy (ASIT or “desensitization”) is an AD treatment wherein extracts of allergens to which the patient is sensitive are injected, in gradually increasing amounts over time, in an attempt to lessen or reverse the hypersensitivity state. The mechanisms by which ASIT produces clinical benefit revolve around modulation of T-cell function and shifting the immune response from a Th2 bias to the more normal Th1 bias. Interestingly, most literature reviews state that ASIT is ineffective or at least questionably effective in human AD; in contrast, ASIT is considered by most veterinary dermatologists to be a highly desirable and useful treatment for canine AD (26,37). ASIT acts on the IgE-mediated component of AD, which may explain why it works only partially or not at all in some patients.

Pharmacologic intervention. Despite the fact that few pathogenetic observations have been extended to canine AD, on the therapeutic side, most new medications for human AD are fairly rapidly subjected to at least empirical trials in dogs. As new therapeutic targets have been uncovered, many new medications for control of allergic diseases in humans have been developed. Unfortunately, very few of these have proven useful in dogs. Leukotriene receptor antagonists, 5-lipoxygenase inhibitors, and the nonsedating antihistamines have not been shown to be useful in animals (38–40). Medications such as pentoxifylline and misoprostol may provide some relief in a few dogs (40,41). The newest treatments for canine AD such as the calcineurin inhibitors cyclosporin A and tacrolimus are aimed at interference with the action of ILs and other cytokines. These drugs are highly effective at suppressing the allergic response and appear to have minimal adverse effects even when given over a long period of time (42,43). Cyclosporin A administered at 5 mg·kg⁻¹·d⁻¹ successfully controls ~70% of atopic dogs, which is a level of control comparable to treatment with corticosteroids (44). Interestingly, in dogs with AD, improvement of clinical signs of AD was unrelated to cyclosporin blood levels. Concurrent treatment with

ketoconazole, which utilizes the same cytochrome P450 metabolic pathway as cyclosporin, greatly reduces the cyclosporin dose necessary to provide control in other canine diseases such as perianal fistula (45). There is presently optimism that a similar combination drug approach could be used for canine AD to minimize the high cost of cyclosporin therapy. The results of such combination drug trials for canine AD have not yet been reported.

Nutritional intervention. Nutritional considerations in AD used to be limited to the role that food hypersensitivity may play in a few patients. However, in the future it may be possible to influence the course of the disease with diet even in the absence of food allergy. It is clear that supplementation of the diet with fatty acids, for example, can change the lipid composition of the canine epidermis (46). In theory, this principle could be used in an attempt to augment compromised cutaneous barrier function. It may even be possible to prevent expression of the atopic phenotype in genetically predisposed individuals. For example, perinatal administration of probiotics such as *Lactobacillus* cultures to human infants at genetic risk for development of AD substantially reduces the risk that clinical disease will develop (47).

We have seen that evidence in the aggregate strongly suggests that AD (at least in humans) is the clinical result of a constellation of pathogenetic mechanisms that may differ from patient to patient and over time in the same patient. Broadly speaking, IgE-mediated hypersensitivity may be involved in the largest subset of patients, yet there is another subset for which such involvement cannot be documented. A variety of keratinocyte defects have been substantiated and certainly influence the course of the disease. Polymorphism in regions of the genome key to the inflammatory response contribute to the patient's clinical picture. Secondary infections modify or augment the inflammatory response, which may change over time. No wonder, then, that AD represents a substantial diagnostic and therapeutic challenge over a patient's lifetime, and that no single treatment is universally effective. Advances in our understanding of this complex disease will no doubt continue to provide us with additional solutions in the future.

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