

Atopy: New Targets and New Therapies

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In the past decade in human medicine, atopy and atopic dermatitis (AD) have been the foci of great research effort because of their increased incidence, lifelong and complex nature, and therapeutic challenges. In veterinary medicine, canine AD has represented a similar challenge for clinicians and researchers. A special Task Force of the American College of Veterinary Dermatology was charged with the mission of reviewing the existing literature and providing clinicians with guidelines for diagnostic criteria and treatment options. From that work, it became evident that there are more questions than answers and that we are just now beginning to unfold the complex, variable, and dynamic nature of this disease. It became obvious that previously accepted definitions and dogmas are insufficient to explain clinical observations and that a more comprehensive approach is necessary to understand and manage our patients successfully [1].

IgE, TYPE I HYPERSENSITIVITY, AND ITS MODULATION

Atopy was traditionally defined as type I hypersensitivity to inhaled allergens. It is now evident that although IgE-mediated reactions play an important role in the capture of the allergens and in the effector phase [2], additional abnormalities are necessary to explain atopy and AD fully [3]. This is supported by several observations. Clinically, antihistamines have limited efficacy, although it could be argued that the limited response may be caused, at least in part, by an inappropriate extrapolation of doses [4,5]. Little information is available regarding the pharmacokinetics of these drugs in small animals; therefore, most of the doses have been extrapolated from human medicine. It is interesting to note that the limited response to antihistamine therapy is not limited to the first-generation antihistamines but has also been reported with the second-generation drugs, such as cetirizine. In one recent study, cetirizine satisfactorily reduced pruritus in only 18% of AD patients when used at a daily

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dose of 1 mg/kg [6]. Similarly, partial success has been reported with other treatments that have focused on type I hypersensitivity, such as leukotriene inhibitors [7] and essential fatty acids [8].

To question the absolute role of IgE in canine AD further, past attempts to establish a model using high-IgE dogs had limited success [9] and allergy testing focused on the detection of IgE is not able to differentiate between normal and affected individuals [10]. Although some of these findings could be explained by the complex nature of IgE [11,12] and speculation that different isoforms of IgE might have different pathogenetic potential, it is also reasonable to consider that additional mechanisms may play a decisive role in the pathogenesis of AD. A subtype of AD, called “intrinsic” or “nonallergic” AD, has been recently identified in which an IgE response is not detectable [13]. Affected individuals have all the other clinical features of AD.

Despite these controversies, modulation or abolition of the IgE component of the disease is still an appealing strategy to control, at least in part, symptoms associated with AD. Great effort has been placed on testing anti-IgE therapies in people [14,15]. Recently, omalizumab, a humanized IgG1 monoclonal antibody against IgE, has been shown to be effective in the treatment of allergic asthma and allergic rhinitis [16]. Omalizumab recognizes and masks an epitope in the CH3 region of IgE responsible for binding to the high-affinity FcεR on mast cells and basophils. Omalizumab markedly reduces serum levels of free IgE and downregulates IgE receptors on circulating dendritic cells. These findings suggest that blocking IgE may also inhibit more chronic aspects of allergic inflammation involving T-cell activation. Although most of the research has focused on respiratory diseases [17], anti-IgE therapy may also have a role in AD, especially in those individuals in which an IgE response is detectable. Anti-IgE therapy is currently being explored in dogs, and vaccination with an anti-IgE peptide was demonstrated to decrease total IgE [18].

LYMPHOCYTES, CYTOKINE AND CHEMOKINE IMBALANCES, AND THEIR MODULATION

Current research is now focusing on the role of cytokines involved in the determination of the type of lymphocytic response and the chemokines responsible for the recruitment of inflammatory cells at the site of allergen challenge [19]. Numerous similarities exist between canine AD and the human counterpart [20], and it seems that in dogs and people, cytokine imbalances geared toward a predominant T helper (Th) 2 response play an important role [21]. More specifically, in canine AD, there is overexpression of interleukin (IL)-4 mRNA and reduced transcription of transforming growth factor (TGF)-β compared with normal dogs [22]. In the same study, higher levels of γ-interferon (IFN), TNFα, and IL-2 mRNA were found in lesional compared with nonlesional and healthy skin, whereas there were no significant differences in IL-10, IL-6, or IL-12 transcription. There is also evidence that chemokines, such as thymus and activation regulated chemokine (TARC), play an important role in the recruitment of Th2 lymphocytes in lesional skin in dogs with AD

[23,24]. Finally, in a recent study evaluating atopy patch test reactions, it was demonstrated that early phases of the reactions are characterized by a predominant Th2 response, whereas later reactions are dominated by a Th1 cytokine profile [25]. All these findings support the consideration of treatment options focused on the modulation of the lymphocytic response, such as calcineurin inhibitors [26].

Calcineurin Inhibitors: Cyclosporine, Tacrolimus, and Pimecrolimus

Calcineurin inhibitors have the ability to block most of the allergic reaction by inhibiting cytokine production and modulating T-cell proliferation and activation and antigen presentation [27–29]. Cyclosporine also inhibits eosinophil function and survival [30,31], decreases the number of Langerhans cells in the epidermis, and inhibits the lymphocyte-activating functions of these antigen-presenting cells [32,33]. Cyclosporine is successfully used in human medicine for recalcitrant AD; however, the severity of the adverse effects has limited its use to severe cases that fail more conventional therapies [34].

Several studies have been published in veterinary medicine evaluating the efficacy and safety of cyclosporine in dogs [35]. Oral cyclosporine at a dose of 5 mg/kg was found to have similar efficacy to prednisolone at a dose of 0.5 mg/kg in a 6-week study [36]. The efficacy of cyclosporine therapy is dose dependent [37,38], but monitoring of blood levels has not been found to be of benefit in predicting and monitoring the clinical response of dogs with AD [39].

In a recent retrospective study that evaluated the response of atopic dogs treated with cyclosporine for a minimum of 6 months, it was found that 55% of dogs needed ongoing cyclosporine to control clinical signs of AD, although daily administration was not necessary [40]. In the same study, laboratory abnormalities were detected in 25% of dogs and therapy was discontinued in 45% because of a limited response (22%) or after achieving a clinical response (24%). Adverse effects of cyclosporine at the dose used for AD have been found to be mostly gastrointestinal disorders (eg, vomiting), development of oral growths, and hirsutism, although concerns have been raised regarding the potential of cyclosporine to induce or precipitate the development of lymphoma [41]. It is important to report that a recent retrospective study evaluating the incidence of lymphoma in 32 dogs receiving cyclosporine for a variety of conditions failed to demonstrate development of lymphoma [42]. Dogs received cyclosporine for a period that varied from 2 weeks to 2 years, with an average of 4 months.

Topical calcineurin inhibitors, such as tacrolimus, may be a suitable alternative to cyclosporin, especially in dogs with localized AD. Tacrolimus ointment has been demonstrated to be safe and effective for short- and long-term treatment of AD in pediatric and adult patients [43,44], although studies on rodents have recently raised concerns about the potential for carcinogenicity and prompted warnings about long-term use [45].

Few studies in veterinary medicine have evaluated tacrolimus' efficacy in dogs with AD. In a 4-week, double-blind, placebo-controlled, crossover study,

tacrolimus ointment at 0.1% significantly decreased the severity of symptoms when evaluated by owners and investigators [46]. Dogs with localized disease responded better than dogs with generalized disease. Minimal absorption was observed with topical application, and tacrolimus was detected in the blood of animals receiving the active ingredient. Levels were below the level of toxicity, and no adverse effects were reported. No changes in complete blood cell count and chemistry parameters were detected.

In another single-blind study using dogs with localized AD, after 6 weeks of therapy, the percentage reduction from baseline scores was higher for tacrolimus-treated sites than for placebo-treated sites [47]. In the tacrolimus-treated dogs, lesions decreased by 50% or greater in 75% of dogs. Adverse drug events consisted of minor irritation in some lesional areas treated with tacrolimus. The irritation could possibly be linked to the ability of tacrolimus to activate nociceptive C-fibers [48].

In human medicine, it is reported that pimecrolimus has a similar mechanism of action and safety profile as tacrolimus but less efficacy [49]. Pimecrolimus permeates less through the skin than tacrolimus and has a lower potential for transcutaneous resorption after topical administration, resulting in a lower risk of systemic effects. Pimecrolimus has high anti-inflammatory activity in animal models of skin inflammation, including a model reflecting neurogenic inflammation, but a more favorable balance of anti-inflammatory versus immunosuppressive activity than tacrolimus [50]. To date, no published studies have been conducted to evaluate the efficacy of pimecrolimus in the treatment of canine AD.

Alternative Modalities of Allergen-Specific Immunotherapy

Multiple open studies and clinical observations suggest that allergen-specific immunotherapy (ASIT) is effective in decreasing clinical signs of AD in dogs [51]. ASIT requires repeated subcutaneous injections, and it may take months before clinical improvement can be observed. The success rate of conventional ASIT in dogs has been reported to vary from 50% to 70% [52].

To abbreviate the induction period, rush ASIT protocols have been explored in veterinary medicine [53]. In one study, the safety of a rush protocol was evaluated in 30 dogs with AD [54]. In this study, allergen extracts were administered in increasing concentrations every 30 minutes for 6 hours to a maintenance concentration of 20,000 protein nitrogen U/mL. In 22 (73%) dogs, rush ASIT safely replaced the prolonged induction period (15 weeks) of weekly injections, which consists of increasing concentrations of allergen extract. In 7 (23%) dogs, the induction period was abbreviated to 4 weeks. Of the 8 dogs that developed problems during rush ASIT, increased pruritus necessitated premature cessation of rush ASIT in 7 and 1 developed generalized wheals. The authors concluded that rush ASIT performed at a veterinary hospital is a safe method for treatment of dogs with AD.

Although the exact mechanisms of ASIT in dogs have not been completely elucidated, it has been shown that ASIT causes a shift toward a Th1 bias by

enhancing γ -IFN expression [55]. More specifically, it was found that the ratio of γ -IFN/IL-4 was low in atopic dogs compared with normal controls before the beginning of ASIT. The level of γ -IFN after ASIT increased significantly, whereas that of IL-4 mRNA was not changed.

An alternate method of suppressing Th2 responses is to take advantage of the innate immune response mounted against bacterial DNA. Oligodeoxynucleotides (ODNs) containing sequence motifs centered on unmethylated CG dinucleotides (CpG ODNs) resemble bacterial DNA and, like bacterial DNA, are immunostimulatory [56]. For this reason, these agents may represent a novel therapeutic approach that restores immune tolerance and suppresses Th2 reactions in atopic individuals [57]. Promising results have been reported with the incorporation of CpG adjuvants in ASIT in human medicine [58]. Similarly, complexes of liposomes and noncoding plasmid DNA may be used to increase Th1 responses, in place of CpG oligonucleotides, because they also stimulate the release of γ -IFN [59].

In a recent open study, seven dogs with refractory AD were treated with liposome–nucleic acid complexes mixed with the ASIT [60]. In this pilot study, the dogs underwent a series of six intradermal injections (weeks 0, 2, 4, 6, 10, and 14). The dose of allergen extract used was decreased by 90% from the dose used in conventional ASIT, based on the known prior potency of the liposome–DNA complex vaccines. Owners assessed pruritus using a visual analog scale, and lesion scores were determined using the Canine Atopic Dermatitis Extent and Severity Index. Circulating levels of γ -IFN, IL-4, TNF α , and IL-10 were measured at the beginning and end of the study. Injections were well tolerated in all dogs, and owners reported a significant improvement in pruritus. A significant decrease in IL-4 production was also reported, although the production of other cytokines did not change significantly with treatment. It is important to note that this study was not blind or controlled; therefore, although these results are encouraging, further investigations with larger numbers of dogs in a controlled and blind study are necessary.

Another alternative approach for ASIT is nonspecific immunotherapy, such as that using MS-antigen. MS-antigen is a peptide extracted from the urine of human allergic patients and is marketed in Japan for nonspecific immunotherapy. In one report, this peptide was successfully used in two dogs that had failed conventional ASIT [61]. Increasing doses of MS-antigen were given every 3 to 4 days initially. Frequency was increased to once weekly to once monthly based on the response. Although various mechanisms of action have been postulated for this compound, the exact mechanism is not known. Various immunomodulatory properties have been reported, including decrease of histamine release, increase of cyclic adenosine monophosphate, and suppression of leukotriene D4. Other antiallergic compounds that have been used in human medicine for nonspecific immunotherapy include histaglobin and neutropin. Histaglobin has been reported to downregulate the release of IL-1 β , TNF α , IL-6, and IL-10 in human peripheral blood mononuclear cell cultures [62] and to decrease or resolve symptoms of AD

and rhinitis [63,64]. So far, no studies have been conducted in dogs to evaluate the efficacy of this therapy.

NEUROPEPTIDES AND THEIR MODULATION

Evidence is building on the relation between the nervous system and the immune system and the potential role for stress and neuropeptides, such as substance P (SP), in the pathogenesis of atopy and AD [65,66]. This is confirmed by the increased plasma concentration of SP and the increased expression of SP in lesional skin of patients with AD [67,68]. In a study evaluating the ability of neuropeptides to affect the T-cell cytokine profile, it was found that the effect is not cytokine specific [69].

Only one study has been reported in veterinary medicine investigating SP concentrations in the skin of normal dogs and dogs with AD. This evaluation was combined with an investigation of the clinical efficacy of 0.025% capsaicin lotion as therapy for canine AD in a double-blind crossover study [70]. In this study, SP concentrations in the skin did not correlate with the severity of the pruritus and did not change significantly over time and with treatments. At the end of the study, significant improvement was reported by owners, suggesting that this treatment could be considered as an adjunctive therapy for canine AD. Initial worsening was noted in some dogs in the first 7 to 10 days of therapy, possibly attributable to increased release of SP.

BARRIER FUNCTION, ITS NORMALIZATION, AND REDUCTION OF ALLERGEN EXPOSURE

In human medicine, AD is characterized by dry skin and increased transepidermal water loss. It is hypothesized that a defective extrusion of lipid-containing organelles, the lamellar bodies, results in changes in the chemical composition of the epidermal lipid barrier and increased transepidermal water loss [71,72]. Extensive research has been done on topical and oral fatty acid supplementation that might help to restore a normal barrier function in patients with AD [73].

At this time, it is not known if a defective lipid barrier is also present in atopic dogs. Studies in veterinary medicine have yielded controversial results. In one study, water absorption/desorption values of dogs with AD did not differ from those of normal dogs [74]. In another study, normal and atopic canine skin was evaluated using transmission electron microscopy to determine structural differences within stratum corneum lipids [75]. The deposition of stratum corneum lipid lamellae in atopic canine skin appeared markedly heterogeneous compared with that seen in normal canine skin. When present, the lamellae often exhibited an abnormal structure. The continuity and thickness of the intercellular lipid lamellae were significantly less in nonlesional atopic canine skin than in normal canine skin, suggesting that the epidermal lipid barrier may be defective in atopic canine skin. Additional studies are clearly needed to characterize the biochemical defect and possibly to correct it with nutritional or

pharmacologic intervention. If transepidermal water loss is increased in atopic dogs, oral supplementation with essential fatty acids and topical application of oils may lead to a normalization of the barrier [76–78].

The other consequence of an abnormal skin barrier is increased penetration of allergens. It is now known that epicutaneous exposure of allergens plays a significant role in canine AD patients [79]. Decrease of allergen exposure can therefore be accomplished by frequent bathing. Unfortunately, epicutaneous exposure is not the only clinically relevant route of exposure. A recent study demonstrated that ingestion of the allergen as well as inhalation and epicutaneous exposure is able to trigger allergic reactions [80]. The approach to affected patients should therefore be multifaceted, with topical therapy being one of the multiple strategies adopted to reduce allergen exposure and decrease clinical signs.

For animals with hypersensitivity to house dust mites, in addition to frequent bathing, it may be useful to use products like benzyl benzoate, an acaricide utilized for the control of house dust mites. In a recently published study, the use of benzyl benzoate led to resolution of lesions and pruritus in 48% of cases of canine AD. In addition, 36% of dogs had moderate improvement, with a decrease of pruritus and minimal skin lesions, but still required some medication [81].

NEW STRATEGIES FOR THE FUTURE

Probiotics

Great interest exists in human medicine regarding early prevention and treatment of AD using microbial probiotics. Probiotics can beneficially boost Th1 immune responses and decrease the likelihood of development of AD [82,83]. Cultures of beneficial live microorganisms characteristic of commensal microflora are administered with probiotic functional foods to provide a microbial challenge for the maturation of gut-associated lymphoid tissue of infants. The probiotic effects are attributed to the normalization of the increased intestinal permeability, the balance of gut microecology, the improvement of the immunologic defense barrier (IgA), the downregulation of proinflammatory cytokines characteristic of allergic inflammation, and the increase of Th1 cytokines (eg, γ -IFN) [84,85].

Most probiotic studies have been conducted using *Lactobacillus rhamnosus* (*Lactobacillus* GG), a specific strain safe to use at an early age, which is effective in promoting local antigen and immune responses and able to survive passage through and transiently colonize the gastrointestinal tract.

To date, there are no studies evaluating this therapy in dogs, although it may be a safe and promising adjunctive treatment for young dogs belonging to breeds with high risk of AD.

Cytokine and Anticytokine Therapy

Therapy with cytokines, such as γ -IFN and IL-4 receptor, have been evaluated in rodents and human beings [86,87]. Because of the potential interference with other immunologic functions of the host, this type of therapy has been replaced

with anticytokines. Anticytokines belong to a new family of biologic response modifiers, which interfere with the biologic functions of cytokines [88]. Anticytokines, such as soluble TNF receptors and IL-1ra inhibitors, have been used to control septic shock and autoimmune diseases and represent promising strategies for the control allergic diseases.

Peptide Immunotherapy

Peptide therapy targets T cells directly with short peptides containing multiple T-cell receptor epitopes [89]. Short allergen peptides, native sequences or altered peptide ligands, are attractive for immunotherapy because they are unlikely to contain epitopes for IgE binding or to induce anaphylaxis. Peptide therapy induces a significant dose-dependent decrease in peptide-stimulated IL-4 production consistent with a shift in T-cell phenotype or peptide-specific T-cell tolerance and may represent an attractive future option of alternative immunotherapy in our patients.

Antisense Oligonucleotides

Antisense oligonucleotides (ASOs) are short single-stranded DNA fragments that are targeted against a specific mRNA. The use of ASOs targeted to the transcripts encoding biologically active proteins in the immunology system provides a novel and highly selective therapeutic strategy to obtain the selective blockage of a specific gene [90]. The advantages of ASOs are high specificity and less adverse drug events than typical immunosuppressive drugs. ASO specific for IL-10 has been demonstrated to resolve AD lesions in a mouse model after topical application [91].

Blockade of Recruitment of Cells

E-selectin on dermal postcapillary venules and homing receptors on lymphocytes play critical roles in the migration of effector T cells into inflamed skin. Cutaneous lymphocyte antigen-1 (CLA-1) is particularly important for homing of T cells in the skin. Modifiers of CLA have been tested in mice and successfully prevented migration of lymphocytes to inflamed skin [92,93]. Additionally, inhibitors of chemokines, such as RANTES and TARC, are being developed to block recruitment of leukocytes at the site of allergen challenge [94].

Targeted Therapy Based on Genetic Testing

It is becoming increasingly evident that AD is a polygenetic disease, with multiple genes being considered as candidates. It is hypothesized that different mutations may be present in different patients and that AD encompasses a variety of genetic alterations, although, clinically, they all lead to similar manifestations. DNA microarray studies are presently allowing the simultaneous comparison of thousands of mRNAs that may help to identify the disease-specific pattern of tissue inflammatory responses [95]. In the future, we may be able to identify the mutations responsible for canine AD in the various breeds of dogs, predict the likelihood of development of disease of young animals, recognize the specific mutations of the affected individual, and prescribe more targeted therapy.

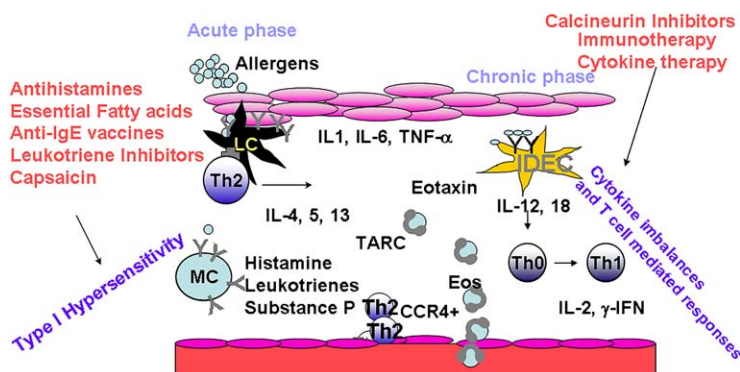


Fig. 1. Proposed pathogenesis for canine AD. Currently, it is accepted that type I hypersensitivity reactions are mixed with T-cell-mediated reactions. Eosinophils are attracted by eotaxin, whereas Th2 cells are attracted by TARC. IDEC, inflammatory dendritic cells; LC, Langerhans cell; MC, mast cell; Th, T helper.

SUMMARY

Because of the multifactorial nature of AD (Fig. 1), the best therapeutic approach is to use combinations of multiple modalities individualized for each patient. Recent research has emphasized the complexity of this disease, indicating how type I hypersensitivity is intermingled with T-cell imbalances and, possibly, abnormalities of the barrier function. New strategies for patient management include modulation of the lymphocytic response through the use of calcineurin inhibitors, potential inhibition of the IgE response through the use of anti-IgE vaccines, and alternative modalities for immunotherapy. Decrease of allergen exposure by frequent bathing, adjunctive antihistamine therapy, and neuropeptide modulation may help in controlling clinical signs. Future strategies may include the use of probiotics, inhibitors of cytokines and chemokines, and a targeted approach based on the genetic mutations of the individual patient.

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