A review of allergen-specific immunotherapy in human and veterinary medicine

Christine Loewenstein* and Ralf S. Mueller†

*Tierärztliche Klinik für Kleintiere, Bereich Dermatologie, Im Langgewann 9, 65719 Hofheim, Germany
†Medizinische Tierklinik, Ludwig Maximilian University Munich, Veterinaerstr. 13, 80539 Muenchen, Germany

Correspondence: Ralf Mueller, Medizinische Tierklinik, Ludwig-Maximilians-Universität, Veterinaerstr. 13, 80539 Muenchen, Germany. E-mail: ralf.mueller@med.vetmed.uni-muenchen.de

Abstract
This article reviews allergen-specific immunotherapy in human and veterinary medicine. Current hypotheses of possible mechanisms of actions are outlined. Indications, success rates, adverse effects and factors influencing outcome of therapy are discussed in humans, dogs, cats and horses.

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Introduction
Atopic dermatitis (AD) is a common disease in dogs1 and humans.2 Approximately 80% of dogs with AD develop non-seasonal clinical signs and require long-term therapy.1 All cases with perennial signs should have adverse food reactions ruled out. Therapeutic options for AD include avoidance of offending allergens, management of secondary bacterial or yeast infections, topical and systemic anti-pruritic drugs such as antihistamines, essential fatty acids, glucocorticoids, or ciclosporin, and allergen-specific immunotherapy (ASIT).

Allergen-specific immunotherapy is the practice of administering gradually increasing quantities of an allergen extract to an allergic subject to ameliorate the symptoms associated with subsequent exposure to the causative allergen (WHO definition).3 Multiple controlled studies have demonstrated that ASIT is effective in the management of human allergic rhinitis, allergic asthma and stinging insect hypersensitivity,3 although its efficacy is more controversial for treatment of AD.4,5 In veterinary medicine, ASIT is a well-established therapy for the management of canine atopic dermatitis (CAD) when relevant allergens have been identified through either intradermal allergy testing (IDAT) or in vitro allergy testing (IVAT). Although there are few randomized controlled trials,6,7 multiple open studies suggest that ASIT is useful in the management of canine,8–11 feline12–14 and equine AD.15–17

The aim of this article is to review the known mechanism of action, efficacy and practical considerations of ASIT in human and veterinary medicine.

Pathogenesis of allergic disease
Allergic diseases result from an exaggerated and often deleterious response of the immune system to specific antigens.18–20 Abnormalities of the skin barrier function probably result in an increased epidermal penetration of allergens and subsequent binding to IgE on the surface of antigen-presenting cells (APCs) such as Langerhans cells in both humans and dogs.21–23

Activated dendritic cells contribute to the generation of allergen-specific CD4+ T helper (T41) cells. Once generated, effector T42 cells produce interleukin (IL)-4, IL-5, IL-9 and IL-13, cytokines with several regulatory and effector functions including the production of allergen-specific IgE by B cells and the development and recruitment of eosinophils.24–26 The degranulation of basophils and mast cells by IgE-mediated cross-linking of receptors is the key event in type I hypersensitivity. Although in humans T42 cells are initially involved in the development of allergic diseases, T41 cells may play an important role in the chronic and effector phase of allergic disease27–29 or decrease allergic inflammation depending on the disease type and stage of inflammation.30 Distinct T41 and T42 subpopulations of T cells counter-regulate each other and play an important role in different diseases.31 In dogs with AD, a T41 response with overproduction of IL-4 mRNA by lymphocytes in lesional skin32 and low expression of interferon (IFN)-γ mRNA by peripheral blood monocytes (PBMC)33 has been reported. A simplified diagram summarizing the pathogenesis of allergic disease is shown in Fig. 1.

Recent studies have demonstrated that peripheral tolerance is crucial for the successful treatment of allergic diseases.34–36 A further subtype of T cells defined as regulatory or suppressor T cells (Treg) have been recently described in humans and mice. These T cells have an immunosuppressive function and a different cytokine profile to either T41 or T42 cells.37–39 Dendritic cells (DC) play an important role for the generation of Treg cells.

Mechanism of action of ASIT in human medicine
The exact mechanism of action by which ASIT achieves clinical improvement is still unclear. Varying allergen preparations, treatment protocols, administration routes and outcome measures reported in different studies make direct comparisons difficult. However, multiple studies...
show that ASIT modifies the responses of APCs, T cells, B cells and the number and function of effector cells that mediate the allergic response.

**Immunotherapy and APCs function in human medicine**

APCs, especially DCs, play an important role in peripheral tolerance and immunity. Immature DCs capture allergens migrate to the T cell region of the local lymph node and then express a partially mature phenotype resulting in the induction of T cell tolerance.56

Their tolerogenic functions depend partly on their maturation status. In the absence of pro-inflammatory signals, as is the case in ASIT, they have a partially mature phenotype and produce co-stimulatory molecules that are intermediate between those of mature and immature DCs resulting in a tolerogenic interaction with lymph node T cells.57

Antigen presentation by partially mature airway DCs induces the formation of regulatory T cells, especially IL-10 producing T regulatory 1 (T\(_{\text{reg}}\)) cells which inhibit subsequent inflammatory responses.60 Antigen presentation by mature DCs leads to the induction of inflammatory T\(_{\text{H}}\)1 cells.51 Several studies have shown that ASIT increases the production of IL-10 by APCs including B cells, monocytes and macrophages.52,54,59 a mechanism that might lead to an increased production of IL-10-secreting T\(_{\text{reg}}\)1-like cells.53

**Immunotherapy and T cell responses in human medicine**

CD4\(^+\) T cells from healthy patients recognize the same allergen epitopes as CD4\(^+\) T cells from allergic individuals.64–66 Studies of T cell responses to both food and airborne allergens showed that these allergens resulted in the induction of T\(_{\text{H}}\)1-, T\(_{\text{H}}\)2- and T\(_{\text{reg}}\)1-cell responses.67 However, the development of allergic disease depends on the quality of the response. In allergic disease, the activity of allergen-specific IL-10-secreting T\(_{\text{reg}}\)1-like cells and CD4\(^+\)CD25\(^+\) regulatory T cells is compromised but can be boosted by ASIT.52,54,67–69

ASIT has multiple effects on T cell response to allergens:

1. Increases the ratio of allergen-induced T\(_{\text{H}}\)1 cytokines to T\(_{\text{H}}\)2 cytokines.70–72
2. Induces epitope-specific T cell anergy.73
3. Generates allergen-specific regulatory T cells that can suppress the responses of effector T cells.54,69
4. Increase the production of cytokines with regulatory activity such as IL-10 and transforming growth factor (TGF-\(\beta\)).53,54,62,74–78

IL-10 has been shown to modulate the function of effector cells in allergic responses. It inhibits the IgE-dependent activation of human mast cells,79 suppresses the production of IL-5 by resting TH2 cells and TH2 cells,80 inhibits the production of granulocyte/macrophage colony-stimulating factor and expression of CD40 by activated eosinophils, and increases eosinophil cell death.81

**Immunotherapy and antibody responses in human medicine**

Patients treated with ASIT showed an increased serum titre of allergen-specific IgG molecules that competed with IgE for the binding of allergen and were therefore termed blocking antibodies.82 However, the relationship between the efficacy of ASIT and the induction of allergen-specific IgG antibodies remains controversial, as studies evaluating a correlation of IgG serum concentration and success of ASIT show conflicting results.83–85 Functional activity rather than antibody concentration may be the relevant parameter.62 Antibody response during ASIT is functionally heterogeneous, probably accounting for the inconsistent data in regard to clinical efficacy.86,87 In addition, allergen-specific IgG response may not be directed against the same epitopes as allergen-specific
IgE influencing the blocking effect. Nevertheless, ASIT-induced IgG has been shown to reduce the IgE-mediated degranulation of mast cells and basophils resulting in a reduction of allergic inflammatory reaction.\textsuperscript{87–89} Furthermore, ASIT-induced IgG inhibits IgE-facilitated allergen presentation to T cells thus leading to a reduction of late phase reaction to allergens\textsuperscript{90} and a decreased number of allergen-specific memory cells that have undergone class isotype switching to IgE.\textsuperscript{91}

Studies on isotype-induced IgG subclasses have shown that specific increases in IgG1 and in particular IgG4 occur.\textsuperscript{92–94} The removal of IgG4 from the sera of allergic patients resulted in a complete loss of binding inhibition of allergen-specific IgE complexes by B cell lines emphasizing its role in this process.\textsuperscript{62}

Furthermore, IgG4 does not fix complement and can inhibit immune-complex formation by other isotypes, giving IgG4 anti-inflammatory characteristics.\textsuperscript{95} In addition, IgG studies have shown evidence for an increased amount of TGF-B-driven allergen-specific IgA following ASIT, indicating that other antibody classes might be involved.\textsuperscript{54}

**Immunotherapy and reduction in skin reactivity**

Numerous changes in the immune reaction to allergen have been documented in humans treated with ASIT and some of these changes appear to be useful markers of an immunological response to ASIT, such as titrated skin prick testing,\textsuperscript{96–97} titrated conjunctival challenge,\textsuperscript{86} titrated nasal challenge,\textsuperscript{96} histamine releasing factor,\textsuperscript{98} PAF from granulocytes,\textsuperscript{100} CD25\textsuperscript{+}CD8\textsuperscript{+} T lymphocytes,\textsuperscript{101} FceRII/CD23\textsuperscript{+} B lymphocytes,\textsuperscript{102} IFN-\gamma mRNA positive cells\textsuperscript{103} and number of mast cells.\textsuperscript{104}

**Conclusion**

Tolerance is a key immunological mechanism in the immune response of healthy individuals to allergens. There is growing evidence for the important role of T_{reg} cells and/or immunosuppressive cytokines in immune responses to allergens during ASIT. T_{reg} cells and their cytokines such as IL-10 contribute to the control of allergen-specific immune responses by suppression of T_{H1} and T_{H2} cells, suppression of allergen-specific IgE, induction of IgG4, IgA or both and suppression of mast cells, basophils and eosinophils.

**Mechanisms of action of ASIT in veterinary medicine**

Compared to human medicine, there is a paucity of studies regarding the mechanism of action of ASIT in veterinary medicine.

**Immunotherapy and T cell responses in veterinary medicine**

After ASIT, peripheral blood mononuclear cells (PBMCs) of dogs with AD stimulated with house dust mite antigen showed an increase in IFN-\gamma mRNA with no change in IL-4 mRNA. Consequently, the IFN-\gamma/IL-4 ratio increased significantly with immunotherapy.\textsuperscript{105} These results suggest a shift to a T_{H1} response in dogs with AD by enhancing IFN-\gamma expression.\textsuperscript{105} IL-4 and IL-5 in the bronchoalveolar lavage fluid of asthmatic cats decreased after rush immunotherapy (RIT) and IFN-\gamma and IL-10 mRNA transcription increased.\textsuperscript{106} Recently, a study evaluating T_{reg} cells and IL-10 concentrations in normal dogs and dogs with AD undergoing ASIT showed a significant increase in both T_{reg} cell numbers and IL-10 concentrations after successful ASIT.\textsuperscript{63}

**Immunotherapy and antibody responses in veterinary medicine**

An increase of IgG following at least 6 months of immunotherapy has been reported in dogs.\textsuperscript{107,108} No correlation was evident between the increase in serum IgG1 concentration and the degree of clinical improvement shown by an individual dog. However, comparison of the increase in serum total IgG1 concentration pre- and post-ASIT in dogs with a good response and those with a fair or poor response revealed a significant difference between the two groups.\textsuperscript{108} A more recent study showed that house dust mite-specific IgE and total IgG antibody concentrations increased during ASIT and subsequently decreased after ASIT was discontinued.\textsuperscript{110} Animals with a poor or equivocal response to immunotherapy had an increase in IgE and total IgG compared to excellent responders.\textsuperscript{110} Other investigators found no significant increases in total IgG or IgG subclass responses in dogs showing a good response to ASIT, suggesting that successful ASIT in dogs is not necessarily associated with the production of blocking antibodies.\textsuperscript{111}

In an experimental model of feline allergic asthma, an increase of specific IgG antibodies was reported 1 and 3 months after RIT with Bermuda grass allergens.\textsuperscript{106} Due to the lack of feline reagents, the antibody subclass responsible for the overall increase could not be demonstrated. No differences in Bermuda grass-specific IgE concentrations were found over time among asthmatic cats treated with RIT, but they had lower IgE concentrations than asthmatic cats that did not receive RIT after 1 and 6 months of therapy.\textsuperscript{106} Bermuda grass-specific IgA concentrations did not change significantly after RIT.\textsuperscript{106} RIT significantly decreased eosinophil-associated airway inflammation in cats with experimental asthma,\textsuperscript{106} a key mechanism in asthma pathology.

**Immunotherapy and reduction in skin reactivity**

Numerous changes in the immune reaction to allergen have been documented in humans treated with ASIT and some of these changes appear to be useful markers of an immunological response to ASIT\textsuperscript{96–104} although they may not necessarily reflect clinical improvement. In veterinary medicine only one study reported a decrease in intradermal allergen test reactions paralleling the clinical improvement in six of 16 dogs receiving ASIT.\textsuperscript{109} However, in this study four of five dogs receiving placebo also lost allergen reactivity.

**Clinical efficacy of ASIT in human medicine**

Many studies have confirmed the efficacy of ASIT in human patients with seasonal allergic rhinitis\textsuperscript{96,112–114} and bronchial asthma due to a variety of allergens.\textsuperscript{115–117} In AD the benefit of ASIT has not been clearly established. Several uncontrolled and open studies suggest that ASIT
may improve the symptoms of human AD, however, the number of controlled studies is small. Two double-blind randomised studies have been reported. One study utilized mixed alum precipitated allergens and reported an improvement in 81% of allergic treated patients compared to 40% in the placebo group, however, the allergen extracts were not standardized and not all patients were treated with the same extracts.

In another double-blind controlled study, children with AD were treated with tyrosine-adsorbed Dermatophagoides pteronyssinus extract or placebo over a course of 8 months. There was no significant difference in clinical outcome between the two groups until the treatment period was extended for a further 6 months, suggesting that an extended period of desensitization may be more effective.

In a more recent study, a house dust mite preparation (D. pteronyssinus/D. farinae) was administered for 1 year to 89 adults with chronic AD and a clinical SCORAD score of at least 40 and allergic sensitization to house dust mites. Patients received one of three different allergen doses at weekly intervals. The SCORAD decreased in the three dose groups in a dose-dependent manner and was significantly lower in the two high-dose groups.

In summary, there is a relative paucity of well-controlled studies investigating the value of conventional ASIT in the treatment of human AD, leaving open the question of whether or not this treatment modality is effective in human AD. There is, however, evidence to suggest that the success rate of ASIT may improve with extended treatment duration and higher doses of allergen extracts.

Clinical efficacy of ASIT in veterinary medicine

Numerous open uncontrolled studies suggest that ASIT is efficacious for the treatment of CAD. The reported success rates with ASIT range from 50 to 100%. The results of these studies seem to be influenced by a number of factors including the allergy testing methodology, the type of allergen and allergen source, induction protocol, dose and concentration of allergen and response criteria.

Studies with similar evaluation criteria such as complete remission, improvement of clinical signs and reduction in medication by more than 50% quoted success rates of 52%, 59%, 60%, 64% and 77%. However, in most studies neither lesion scores, pruritus scores nor the concurrent need of symptomatic antipruritic therapy were significantly lower in the two high-dose groups.

In summary, there is a relative paucity of well-controlled studies investigating the value of conventional ASIT in the treatment of human AD, leaving open the question of whether or not this treatment modality is effective in human AD. There is, however, evidence to suggest that the success rate of ASIT may improve with extended treatment duration and higher doses of allergen extracts.

Factors affecting clinical efficacy in human medicine

Patient selection

Guidelines and indications for immunotherapy have been published by many professional organizations, but guidelines do not represent a consensus report of experts from all parts of the world and vary widely. ASIT in human medicine has been reported to be effective for the treatment of allergic rhinitis, allergic bronchitis, allergic asthma and stinging insect hypersensitivity. It is recommended in patients who have demonstrable clinical response in the management of grass pollen allergy in humans. 127,128

Another double-blinded, randomized study compared conventional and rush immunotherapy using aqueous allergens in dogs with AD. In this study, an improvement of more than 50% in pruritus was observed in 45% and 55% of the dogs, respectively, and in lesion scores in 64% of the dogs in both groups.

All other reports were either open and uncontrolled studies or anecdotal observations and the conclusions need to be validated in controlled studies before they can be accepted. However, despite a lack of evidence based on randomized controlled trials, results of open uncontrolled studies and a wide body of global observation by veterinary dermatologists suggest that aqueous ASIT is clinically efficacious for the treatment of dogs with AD.

In cats, immunotherapy formulated based on positive intradermal tests has only been described in open studies and the reports do not include large numbers of cats. Traditionally, evaluation of intradermal test results in cats has been considered more difficult than in dogs due to the often subtle and short-lived reactions; consequently, many veterinary dermatologists are reluctant to perform intradermal testing in cats. To facilitate the evaluation of skin tests in cats the intravenous injection of 10% fluorescein solution was recommended. ASIT for feline atopy has resulted in success rates ranging from 50% to 75%. Presenting clinical signs in cats undergoing ASIT included feline eosinophilic granuloma complex, miliary dermatitis, self-inflicted alopecia, facial pruritus, and seborrhea with mild pruritus. In a double-blinded study, immunotherapy with flea antigen was found to be unsuccessful in the management of cats with flea allergy dermatitis.

In equine medicine there are few studies on ASIT. Most studies have not been controlled and included only small numbers of horses. One study on Culicoides (Diptera: Ceratopogonidae) hypersensitivity evaluated ASIT in a double-blinded control fashion with poor results. However, in another trial, all 10 horses with Culicoides hypersensitivity improved during immunotherapy, and seven of these horses deteriorated again after cessation of therapy. Differences in climate and study protocols make a comparison of the study results difficult. Most authors reported a 60% to 71% good to excellent response to ASIT based on the results of intradermal testing. Reports evaluating the influence of multiple concurrent positive reactions to insects on the outcome of ASIT show conflicting results.

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evidence of specific IgE antibodies to clinically relevant allergens and whose allergic symptoms warrant the time and risk of immunotherapy. The necessity for immunotherapy depends on the degree to which symptoms can be reduced by medication, the amount and type of medication required to control symptoms, and whether effective allergen avoidance is possible. In human patients, an improved efficacy with ASIT appears to be associated with several factors. ASIT seems to be more effective in asthma patients if it is commenced at a young age and early in the course of the disease and with hypersensitivity to one or only few allergens. The advantages of instituting ASIT early in the disease process include the prevention of chronic inflammation, prevention of further development of severe disease and reduction of the potential for adverse reactions to immunotherapy. A further advantage of the early use of immunotherapy is to prevent the development of sensitization to additional allergens.

Allergen selection
In humans, response to ASIT appears to be allergen-specific. Most of the recent studies have employed single allergen extracts and selected patients on the basis of positive prick tests and in some circumstances additional positive in vitro tests. In humans, ASIT is generally prescribed using single or few allergens. One study compared the efficacy of ASIT in patients with grass allergy and polysensitized patients. Grass pollen-allergic patients improved significantly compared to placebo treatment, whereas the improvement of polysensitized patients was not significant. However, older studies show a positive response to mixed pollen immunotherapy or immunotherapy containing multiple unrelated allergens.

Dose of allergens
The optimal dose of ASIT is defined as the dose of standardized allergen extract that induces a clinically relevant effect in the majority of patients without causing unacceptable side effects and is described in either biological units or in mass of major allergens. In human ASIT, an amount of 5–20 μg of the major allergen is reported to be optimal for domestic mites, cat dander, ragweed pollen and hymenoptera venoms. Early comparative studies with low-, intermediate- and high-dose immunotherapy indicated that groups receiving higher doses of antigen showed significantly fewer symptoms after ASIT and that low-dose allergen immunotherapy was ineffective. Although high-dose therapy may be reported to be more effective, the risk versus benefit suggests that intermediate dose may be preferable because of the lower risk of adverse reactions. In selected patients, an adjustment of the dose may be necessary.

Allergen mixtures
In human ASIT, extracts consist either of a single allergen, mixtures of unrelated allergens or related cross-reacting allergens. If mixed-allergen immunotherapy is prescribed, each allergen group should be present in approximately similar amounts and botanical cross-reactivity must be considered. Clinically, significant cross-reactivity of allergens in humans has been demonstrated in the conifer family and among grasses. Two problems that may occur with the use of allergen mixtures are excessive dilution and loss of allergenicity. Excessive dilution can lead to suboptimal dosing and more rapid allergen deterioration while loss of allergenicity can occur as a result of the innate enzymatic activity of some allergen extracts.

Extracts of fungal and whole body insects contain proteases that are capable of degrading other allergens. It is recommended to not mix cockroach or fungal extracts with pollen extracts, unless the diluent contains 25–50% glycerine. Glycerin seems to inhibit the activity of some proteases and may also retard loss of potency of an allergen extract and seems to be most effective at a concentration of 50% but its use is limited by the pain that accompanies injection. Loss of potency is accelerated by higher temperatures, therefore, allergen extracts should be kept refrigerated.

Administration of ASIT
In the majority of studies in human medicine, ASIT has been administered by subcutaneous injections of allergen. In classic ASIT, gradually increasing dosages of the allergen extract are administered (induction period) until the individual maximum dose is reached (maintenance period). The induction phase is conventionally achieved by twice weekly to every 2 weeks injections of allergen extracts. Accelerated allergen immunotherapy schedules such as cluster and RIT have been used in order to rapidly reach the maintenance dose and to achieve clinical improvement sooner. In cluster immunotherapy, the number of injections is increased to two to three injections per day of treatment once weekly and in RIT to eight injections over a period of 3 days, up to eight in a single day. Cluster immunotherapy and RIT seem to be an effective alternative to conventional immunotherapy.

Duration of ASIT
In humans, the duration of immunotherapy required to maintain improvement in clinical signs is unknown. The clinical benefit may increase with continuation of therapy over several seasons. The effect from a brief course of immunotherapy may be rapidly lost, whereas after a longer treatment course, remission may persist even after cessation of injections. For the patients responding to treatment, many clinicians advise to continue ASIT until the patient is asymptomatic or has demonstrated a substantial improvement in clinical signs over a period of several years. Two studies have examined the persistence of clinical improvement after cessation of immunotherapy with grass pollen extract. Immunotherapy for 3 to 4 years induced prolonged clinical remission accompanied by a persistent alteration in immunological reactivity. There was a progressive increase in the number of patients reporting recurrence of clinical signs after discontinuation of ASIT, reaching 31% by the third year. In another study, some patients maintained their level of improvement over a 12- to 96-month period after discontinuing immunotherapy with house dust mite extract. However, more than half of the patients had a recurrence of clinical
Adverse reactions

Besides the beneficial effects of immunotherapy, localized or systemic adverse effects and rarely fatal outcomes are also possible.\textsuperscript{192,193}

In humans, local reactions to ASIT are characterized by erythema, swelling and pruritus and typically appear within hours of the injection. They were not found to be predictive for systemic reactions\textsuperscript{194} and may not require dose adjustments.\textsuperscript{195} Nevertheless, most allergists adjust the dose after local reactions because of discomfort which may lead to patient noncompliance.\textsuperscript{196}

The most common systemic reactions were rhinitis, asthma and anaphylaxis,\textsuperscript{197} typically within 30 min after the injection of allergen extract.\textsuperscript{190,198} Therefore, patient monitoring in the clinician's office for 20 min and in high-risk patients for 30 min after injection is recommended.\textsuperscript{193}

The potential risk of ASIT is small. Studies with high numbers of patients monitored over a period of 1 to 13 years reported a rate of systemic reactions in 2.1–2.9\% of patients.\textsuperscript{198,199} Reactions were more common during the induction phase and not only restricted to the patient's pollen season,\textsuperscript{198,199} although an increased risk during this time has been suggested.\textsuperscript{193}

Patients with asthma are at greater relative risk to have a fatal reaction with allergen immunotherapy than patients with rhinitis.\textsuperscript{192,193,197} Additional risks are dosage errors on first injection from a new vial of extract, use of beta-adrenergic blocking agents and home administration of immunotherapy.\textsuperscript{193}

A review on the efficacy and safety of accelerated immunotherapy reported a wide range of systemic reactions but concluded that in selected patients it may provide a rapid alternative to conventional build-up schedules without a significant increase in risk.\textsuperscript{187}

In order to reduce systemic reactions to immunotherapy, premedication with oral corticosteroids, ketotifen and theophylline,\textsuperscript{200,201} or oral corticosteroids and a combination of H\textsubscript{1} and H\textsubscript{2} antagonists has been suggested.\textsuperscript{202} The administration of loratidine 2 h before each allergen injection was reported to reduce systemic reactions during cluster immunotherapy,\textsuperscript{203} Montelucast can be useful in the prevention of local reactions.\textsuperscript{204}

Re-evaluation of patients during immunotherapy

Many patients discontinue ASIT on their own choice due to the long treatment duration and perceived inconvenience.\textsuperscript{205–207} A closer contact with the patients receiving injections may help to reduce non-compliance.\textsuperscript{207}

Factors affecting clinical efficacy in veterinary medicine

Patient selection

Allergen-specific immunotherapy is indicated in dogs with a diagnosis of AD where intradermal testing or allergen specific serology has enabled the identification of allergens, where allergen contact is unavoidable and symptomatic anti-inflammatory therapy is either ineffective, or associated with unacceptable side-effects.\textsuperscript{206} If symptomatic therapy is impractical, ASIT may be indicated even in seasonal disease of short duration.\textsuperscript{209}

Many of the previous studies have reported that the efficacy of ASIT in dogs with AD can be variably affected by several predictive factors including the age at the onset of the disease, the age at commencement of ASIT and the duration of the disease.

Most studies are in agreement that age at disease onset,\textsuperscript{12,14,18,23} age at commencement of immunotherapy\textsuperscript{12,13,18,20,210} and the duration of disease\textsuperscript{18,23,211} prior to therapy do not influence the success rate of ASIT. Only few studies indicate a tendency for lower response in older dogs,\textsuperscript{21,23,212} or dogs having clinical signs for more than 60 months.\textsuperscript{21,213}

The majority of reports support a role for allergy testing and ASIT in older atopic dogs because they are less likely to develop new allergies that could interfere with the efficacy of immunotherapy.\textsuperscript{109,214} At this point, it seems unlikely that age of the dog or disease duration significantly alters the success rate of ASIT.

Another predictive factor that has been cited in various studies is the influence of seasonality of clinical signs on the outcome of canine ASIT.\textsuperscript{13,23,214} One study suggested that dogs with non-seasonal signs respond better to ASIT,\textsuperscript{214} contrasting with two other studies that reported either worse response,\textsuperscript{13} or no seasonality influence to the response rate.\textsuperscript{23}

Some authors have suggested that dog breed and sex may influence the success of canine ASIT. Boxer, West Highland white terrier and golden retriever have been reported to be less responsive to ASIT.\textsuperscript{109,215} However, other studies did not confirm these results.\textsuperscript{27,213,214} One study\textsuperscript{214} found females to have a higher percentage of ‘good to excellent’ responses than males. In contrary, a different study found no gender difference\textsuperscript{14} and another reported a trend for males to respond better than females.\textsuperscript{23}

Lastly, several studies have suggested that the number and the type of allergens to which the patient is hypersensitive may influence the outcome of canine ASIT. Some studies reported that dogs treated with larger numbers of allergens had a lower response rate,\textsuperscript{23,109,214,216} and that ASIT with 10 or less allergens were more effective\textsuperscript{20} while other authors reported the highest clinical response in dogs treated with 11 to 20 allergens.\textsuperscript{126} More recent studies showed no correlation between the efficacy of ASIT and the number of allergens.\textsuperscript{18,213}

There were also conflicting results evaluating specific types of allergens. In some studies pollen-hypersensitive dogs showed better results than dogs with other types of hypersensitivity.\textsuperscript{12,27} Conversely, other investigators did not show any difference in the response to allergen extracts containing either house dust mite antigens or pollen antigens\textsuperscript{213} or different types of antigens.\textsuperscript{18}

It is known that mould proteases degrade pollen allergens when they are stored in the same vial.\textsuperscript{192,213} However, whether this degradation has clinical consequences is controversial. The success rate of ASIT containing mould antigens in one study was much less than that containing pollen or dust mite allergens.\textsuperscript{18} In
a subsequent report using identical allergens and ASIT treatment protocols, mould allergens were dispensed in separate vials and the success rate of ASIT containing mould allergens increased and was comparable with that of other types of allergens. In contrast to human medicine many veterinary dermatologists and laboratories still formulate therapeutic extracts containing both mould and pollen antigens together and double-blinded prospective studies are needed to confirm the relevance of antigen separation in veterinary medicine.

Most of the studies evaluating factors affecting clinical efficacy of ASIT were anecdotal and observatory in nature. Further validation in controlled studies is needed.

**Allergen selection**

The clinical response to ASIT in dogs appears to be allergen specific and allergens must be selected on the basis of the patient’s clinical history and results of the intradermal testing or allergen-specific serum IgE testing. A thorough work-up and exclusion of other potential diseases is essential for the clinical diagnosis of AD before allergy testing and ASIT are initiated, as intradermal tests of normal dogs have shown positive reactions.

In a double-blinded study, dogs treated with a non-specific set of allergens exhibited a median improvement in their clinical score of 18%, whereas dogs treated with allergen specifically selected on the basis of intradermal testing showed a median improvement of 70%.

Many reports on the efficacy of ASIT based on results of allergen-specific IgE serology have suggested that the outcome is similar to those based on intradermal test results although, this was not a consistent finding. The efficacy of ASIT may be potentially improved if the results of both allergy tests are combined.

In cats treated with ASIT based on in vitro testing, 75.3% showed an improvement of at least 50%.

In horses problems with accuracy and repeatability of ELISA tests have been reported. A small uncontrolled study including horses with atopic urticaria reported a poor outcome. In a double-blinded study, dogs treated with allergen specifically selected on the basis of intradermal testing showed a median improvement of 70%.

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Dose of allergens

In human medicine, the allergen dose for optimal clinical response has been defined for many standardized allergens but no standardized allergens are available in veterinary medicine. Furthermore, previous studies and references have reported the concentration of allergens in different units and often failed to specify the absolute concentration of each allergen in the final mix making the comparison between reports difficult. It is convention to use a maintenance vial with a total concentration of between 10 000 to 20 000 PNU mL⁻¹. However, there are some studies that compare high- or low-dose immunotherapy protocols with the conventionally used doses. In one open study, an increased response to ASIT was demonstrated with higher allergen doses although the efficacy of low-dose protocols has been demonstrated and the need for reduced allergen concentrations in small dogs has also been suggested.

In a small pilot study in France using two concentrations of house dust mite extract for ASIT, there was a greater reduction in the medication score within 1 year with the typical dose protocol of 115 reactivity indices (IR) than with a higher dose protocol of 600 IR. In contrast, in another study, low-dose and standard-dose ASIT were compared and no significant difference was noted in the occurrence of secondary infections or glucocorticoid requirements of the patients included in study.

**Administration of immunotherapy**

In dogs, subcutaneous injections are the standard route of administration. Oral allergen treatment did induce tolerance in dogs sensitized to ovalbumin but not in two dogs sensitized to house dust mite antigens.

The optimal dosing interval for both loading and maintenance allergen injections has not been established and may be dependent on the patient. Loading protocols with increasing doses and injection intervals of 2 to 7 days and maintenance protocols with treatment intervals between 5 and 20 days have been suggested. Other studies have reported the interval between maintenance injections ranging from 3 weeks to 5 months or 1 to 12 months. One author suggested that the protocol should be modified based on the patient’s response to the injected allergens in order to optimize success. In dogs with persistent clinical signs after 6 months of immunotherapy, the concentration of the extract was decreased. If this modification resulted in resolution of pruritus, ASIT was continued without further change. In dogs with a partial decrease of symptoms, further reductions of extract concentration were performed until remission was achieved. In individual patients, the concentration of subsequent injections was again increased if the previous reduced concentration resulted in an increase of pruritus. Adjusting the injection frequency and dose of allergen extract to the patient’s requirements is, thus, important in optimizing therapeutic efficacy.

Short and intense protocols of immunotherapy (rush protocols) have been reported in veterinary dermatology and have resulted in a rapid clinical response. However, in RIT using the intraderal route of injection a higher incidence of adverse reactions in terms of severe pruritus and urticaria than in conventional protocols has been observed, which required premature discontinuation of the RIT in 23% of the cases. In a subsequent double-blinded, randomized, controlled study comparing conventional ASIT with RIT using subcutaneous injections, none of the dogs undergoing RIT showed adverse effects. However, close monitoring of hospitalized patients is still recommended during the day of RIT.

Another approach to increase the efficacy of ASIT was the addition of immunostimulatory molecules. In a recent
pilot study, immunotherapy using a combination of allergens and immunostimulatory liposome-plasmid-DNA complexes was evaluated in dogs which had previously unsatisfactory responses to at least 12 months of conventional ASIT. After six intradermal injections over a period of 14 weeks, a significant improvement in pruritus scores and a significant decrease in IL-4 production suggested a beneficial effect, but further investigations in larger numbers of dogs are warranted.

In a pilot study with four cats, a RIT protocol was conducted to reach maintenance therapy. All allergen extracts were administered subcutaneously in the dorsal neck area at increasing PNU’s every 30 min for five hours to an average maintenance of 7500 PNU mL⁻¹. The purpose of this study was to determine a safe protocol for RIT in atopic cats; therefore, clinical efficacy was not assessed.

Additional controlled prospective studies with higher number of animals are needed to determine if RIT is preferable to conventional ASIT.

**Time to efficacy**

The time to maximal clinical benefit and total duration of ASIT before determining treatment efficacy are currently unknown in veterinary medicine. These factors, however, have significant impact both on the patient’s well-being and on the owner’s compliance. The studies utilizing aqueous and alum precipitate allergens report varying rates of improvement from 2 to 5 months while others cite 6 to 9 months. Willemsen suggested that evaluation of response to ASIT with alum-precipitated allergens could be restricted to 9 months as dogs not responding by that time are unlikely to respond at a later date. In another study, 11% of the dogs receiving ASIT with aqueous allergens only responded after receiving therapy for more than 8 months. In a double-blinded study comparing RIT with conventional ASIT, the mean time to maximal improvement was 6.8 versus 9.2 months, respectively; however, this difference was not statistically significant.

In cats and horses, response to therapy was seen after 1 to 4 months and 8 to 12 months, respectively.

**Duration of ASIT**

The long-term efficacy of ASIT in dogs, cats and horses with AD has not been evaluated in any controlled studies. In dogs, open uncontrolled studies with unspecified or variable follow-up periods have shown sustained clinical improvement after discontinuation of ASIT in 23% to 35%, 12%, 6% and 4% of the dogs, respectively. There are no reported data about long-term efficacy of ASIT in the cat and horse.

**Adverse reactions**

In dogs, the most common adverse reaction is increased pruritus after administration of increasing concentrations of immunotherapy. The incidence of adverse effects with ASIT ranged from 5% to 50% on standard ASIT protocols. However, in these studies, concurrent anti-pruritic or anti-inflammatory drug treatment was not permitted during immunotherapy. The clinical deterioration with injections of allergen extracts may indicate that the animal’s maximum tolerance of allergens has been exceeded.

Subsequent individualized dose modification resulted in an increased success rate. In a blinded placebo-controlled study, three of 27 dogs treated with alum-precipitate allergens and two of 24 treated with alum-containing placebo suffered from generalized pruritus, indicating that at least part of the pruritus could have been due to the adjuvant.

Systemic reactions have been reported to occur in about 1% of the dogs and included weakness, depression, anxiety, sleepiness, panting, hyperactivity, diarrhoea, vomiting, urticaria/angioedema, collapse and anaphylaxis. In cats and horses there are few data available on the incidence of adverse reactions, however, one of the authors (RM) has seen fatal anaphylaxis in a small number of cats. In a small study on RIT two of four cats developed mild pruritus and a dermal swelling on the dorsal neck one week later.

Localized injection site reactions in dogs, cats and horses are rare and normally do not require modification of the treatment protocol.

**Effects of medication**

The effects of concurrent glucocorticoid therapy during ASIT have not been evaluated in the dog, cat or horse but such interaction may be of significance. Some authors suggest that ASIT still may be effective if prednisolone is administered in low doses or on alternate days, whereas other authors recommend the avoidance of glucocorticoids in the induction phase of ASIT. One study reported alternate-day prednisolone therapy during induction phase of ASIT with no apparent loss of efficacy. Besides possible suppression of desirable mechanisms of immunotherapy, glucocorticoids may mask improvement of clinical signs as well as adverse reactions requiring a modification of the treatment protocol.

In human medicine, ciclosporin does not seem to significantly inhibit intradermal reactivity in dogs when given short-term at a dose of 5 mg/kg daily. However, long-term effects on ASIT are not known.

Because RIT is associated with a relatively high incidence of systemic reactions in humans, patients are routinely pre-medicated with a myriad of medications including glucocorticoids, antihistamines and leucotriene inhibitors. With the exception of leucotriene inhibitors similar medications have been utilized in canine and feline pre-medication protocols.

**Re-evaluation of patients during immunotherapy**

Owner compliance plays an important role when using ASIT in veterinary medicine. Up to 49% of owners discontinued therapy without consulting the clinician or were lost to follow-up in published studies. The main reason for cessation of ASIT is lack of improvement. Recurrence of clinical signs during immunotherapy is often associated with flare factors such as bacterial pyoderma and Malassezia dermatitis, or the development of new allergies. Modification of the treatment protocol (frequency, amount of injected allergens, etc.) is frequently needed to optimize success rates. Recognition and management of flare factors, newly developing allergies and expertise in individualizing immunotherapy are essential in achieving the high success rates published.
Summary
Allergen-specific immunotherapy has been shown in well-controlled studies to be an effective treatment for allergic diseases in humans caused by environmental or venom allergens. In veterinary medicine, ASIT is used in dogs, cats and horses for the management of AD but there is a clear paucity of randomized, controlled scientific trials to determine the true efficacy of ASIT and the optimal allergen doses and frequency of injections. Further studies are required to evaluate this therapy further.

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Résumé Cet article décrit l’immunothérapie spécifique d’allergène chez l’homme et en médecine vétérinaire. Les hypothèses actuelles des mécanismes potentiels d’action sont décrits. Les indications, les taux de succès, les effets secondaires et les facteurs influençant le succès de cette thérapeutique sont décrits chez l’homme, le chien, le chat et le cheval.

Resumen Este artículo revisa la inmunoterapia específica de alergeno en medicina humana y veterinaria. Se comentan las hipótesis actuales de los posibles mecanismos de acción. También se discuten las indicaciones, índices de éxito, efectos adversos y factores que influyen el resultado de la terapia en humanos, perros, gatos y caballos.