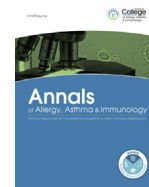




Contents lists available at ScienceDirect



Review

Dog allergen immunotherapy: past, present, and future

Derek M. Smith, MD; and Christopher A. Coop, MD

Department of Allergy/Immunology, Wilford Hall Ambulatory Surgical Center, San Antonio, Texas

ARTICLE INFO

Article history:

Received for publication October 1, 2015.

Received in revised form November 25, 2015.

Accepted for publication December 2, 2015.

ABSTRACT

Objective: To review the published medical literature on dog allergy immunotherapy and discuss prior clinical trials, important allergens, extract specifics, and potential future treatment options for dog allergy relevant to the clinical allergist.

Data Sources: MEDLINE search was performed using the terms *dog*, *immunotherapy*, and *allergy* limited to human studies from any period. Articles cited in selected studies also were reviewed for appropriateness of inclusion into this review.

Study Selections: Publications were included that were original research and fit the topic of dog allergen immunotherapy, specifically articles that investigated prior effectiveness and safety of dog allergen immunotherapy, dog extracts, identification of dog allergens, and current prescribing trends among allergists.

Results: Two hundred fifteen articles were initially identified and 60 were reviewed in complete detail for inclusion in this review. The primary focus was placed on the 17 clinical trials that investigated the safety and efficacy of dog immunotherapy and the 19 studies that explored and defined the complex allergenic profile of dog extracts.

Conclusion: The medical literature on the use of dog extract immunotherapy in patients with hypersensitivity to dog shows poor and conflicting results of clinical efficacy, which has been attributed to poor-quality extracts and the inherent complex allergenic profile of dogs that remains without a clearly dominant allergen.

Published by Elsevier Inc. on behalf of the American College of Allergy, Asthma & Immunology.

Introduction

According to the American Pet Products Association, dogs currently live in more than 54.4 million US homes, and studies have shown that Can f 1 is nearly ubiquitous in public places.¹ Domesticated dogs initially fulfilled a pragmatic role for people, for protection or for agricultural use, but now serve a more emotional role of companionship. With this paradigm shift, dogs have moved indoors and constant exposure is associated with the development and exacerbation of allergic disease. For the patient with dog allergy, avoidance is clearly the best treatment modality, but this frequently does not occur even in settings beyond occupational allergy. The sensitization rate is reported as high as 10% of all people in Westernized countries,² and this rate varies by country, time period, and atopic predisposition.^{3–5} As such, great efforts have been made in determining ways to minimize dog allergen levels in the home without removing the dog. Specific trials have looked at frequent washing of the dog,⁶ using high-efficiency particulate air-filtered vacuum cleaners,⁷ using high-efficiency particulate air

room air filters,⁸ and even breeding “hypoallergenic” dogs⁹ or consistently altering their natural hair length.¹⁰ All these methods can lower allergen levels but have failed to resolve the issue of dog allergy. Therefore, the allergist is presented with the option of recommending dog immunotherapy to help ameliorate patients’ symptoms. This article reviews the current evidence on which to base this decision.

Early Clinical Studies

After the advancement in protein extraction methods in the early 1960s, it was hypothesized that the role of immunotherapy would expand far beyond pollens in the treatment of allergic disease. Soon after, reports were published about symptomatic improvement for patients with dog allergy after immunotherapy with the newest form of dog extract. The oldest report of successful dog immunotherapy was a presentation by Gould¹¹ in 1963, who reported a case series of 11 patients with dog allergy whose symptoms were alleviated after being treated with dog extracts. In 1967, Tuft and Torsney¹² reported that 13 of 17 patients (76%) with dog allergy had improved tolerance to dog exposure after immunotherapy, and Scherr¹³ reported improved tolerance in 25 of 27 patients (93%) treated with dog extracts. However, no placebo injection or blinding controlled these trials and the improvement was based solely on subjective symptom scores.

Reprints: Derek M. Smith, MD, Allergy/Immunology, 2200 Bergquist Drive, Suite 1, Lackland AFB 78236; E-mail: derek.smith.3@us.af.mil.

Disclosures: Authors have nothing to disclose.

Disclaimer: The opinions or assertions herein are the private views of the authors and are not to be construed as reflecting the views of the Department of the Air Force or the Department of Defense.

<http://dx.doi.org/10.1016/j.anai.2015.12.006>

1081-1206/Published by Elsevier Inc. on behalf of the American College of Allergy, Asthma & Immunology.

Although these early results were encouraging, clearly there was a need to improve the quality of evidence to support the use of dog allergen immunotherapy. As more data accumulated, the excitement for the effectiveness of this therapy began to wane. In 1968, Brown and Wolfe¹⁴ prospectively compared immunotherapy with alum-precipitated dog extracts with the previous standard aqueous extracts in 50 patients with dog allergy. The 2 groups showed less convincing results because only 11 of 38 patients (29%) in the alum group and 4 of 12 patients (33%) in the aqueous group experienced any subjective improvement. No comparable control group was available to evaluate for potential placebo effects. To refute these negative results, Tuft and Torsney¹⁵ reported additional information in 1976 regarding their continued experiences with dog immunotherapy with an additional 67 patients. A total of 62 of 84 patients (74%) subjectively reported increased tolerance to dogs, but there were no objective end points or placebo control group with which to compare these results.

First Double-Blinded Placebo-Controlled Trial

To aid in clarifying these conflicting data, the first randomized, double-blinded, placebo-controlled trial regarding the effectiveness of dog immunotherapy was published in 1984 and involved 27 children with asthma and dog allergy. Fifteen patients were placed on dog dander extracts and 12 received placebo injections containing histamine. The patients' responses were evaluated by bronchial and conjunctival provocation challenges and symptom scores. After 12 months of immunotherapy, 12 of 15 patients (80%) had improved tolerance to the conjunctival challenge compared with 2 of 12 (17%) who received placebo, 6 of 15 patients (40%) had improved tolerance to dog allergen bronchoprovocation compared with 2 of 12 (17%) who received placebo, and 8 of 15 patients (53%) had improved by subjective symptoms compared with 6 of 12 (50%) who received placebo. The only statistically significant difference for immunotherapy compared with placebo was noted to the conjunctival challenge because there was no difference in symptom scores.¹⁶ Early laboratory methods of comparing specific immunoglobulin (Ig) E did not significantly change, but specific IgG increased significantly. The overall lack of improvement in asthma was postulated to be due to poor extract quality or confounding allergens, such as cat, for which this study did not control.¹⁷

The "Landmark" Dog Immunotherapy Trial

After further refinement in pet dander extracts, the landmark trial in dog allergy immunotherapy occurred in Denmark and initial results were published in 1986. This study compared 7 patients with asthma and dog allergy (and 15 with cat allergy) with 17 patients receiving placebo injections. The primary outcomes of investigation were symptom scores, bronchoprovocation testing reactions to dog allergen extract and histamine,¹⁸ skin prick testing reactions, specific IgE level, and specific IgG and IgG4 levels.¹⁹ At the end of 12 months of therapy, 4 of 7 patients (57%) with dog allergy reported improvement in symptoms and could tolerate roughly 3 times more conjunctival allergen (determined not to be statistically significant) but could not tolerate additional histamine levels by bronchoprovocation. Skin prick testing to dog extracts showed significantly decreased wheal diameter throughout the study in all patients who received immunotherapy. Specific IgE levels increased significantly in all patients receiving cat or dog immunotherapy during the first 9 months of therapy but began to decrease in the following 3 months in adults, whereas they continued to increase in children, which could be secondary to the effect of immunotherapy or natural exposure to allergen. Specific IgG and IgG4 levels also significantly increased in all patients. Importantly, no correlations between any laboratory

measurements and symptom scores or allergen-specific bronchoprovocation testing were noted.

At the end of the initial 12 months, the blinding code was broken and the 4 patients with dog allergy receiving placebo were offered immunotherapy for 3 years (a total of 11 patients). After 2 years of immunotherapy, no significant change in outcomes was noted compared with just 1 year of treatment.²⁰ At completion of 3 years, symptom scores improved in 4 of 9 patients (44%) and allergen-specific bronchoprovocation testing (mean provocation concentration that caused a decrease in forced expiratory volume in 1 second of 20% of 0.2 mg/mL) and skin prick testing (mean wheal from 5.9 to 4.2 mm) results significantly improved, whereas nonspecific histamine bronchoprovocation testing failed to show a meaningful decrease in bronchial hyperreactivity. All improvements were noted in the first year and failed to improve further with additional treatment duration. There was no significant change in specific IgE when comparing pretreatment with post-immunotherapy values, and the diminutive increase in allergen-specific bronchoprovocation tolerance was unlikely to be clinically relevant.²¹

For patients who had received 3 years of dog immunotherapy, a 5-year follow-up evaluation was completed. Of the final 11 patients who had received dog immunotherapy, 9 patients responded to the symptom questionnaire, 6 agreed to the allergen challenge, 7 agreed to the histamine challenge, and 7 agreed to additional blood testing for IgE and IgG4. Three of the 4 patients who noted symptomatic improvement at the end of the 3-year study maintained this improvement 5 years later, whereas 1 patient noted worsening after discontinuing immunotherapy. Five of the 6 patients who underwent allergen-specific bronchoprovocation testing experienced an increase in sensitivity 5 years after the cessation of treatment compared with their bronchoprovocation testing results at the end of 3 years of immunotherapy. Specific IgG4 had decreased and specific IgE remained low at levels comparable to the end of 3 years of treatment. There was no correlation with the changes in immunoglobulin levels, bronchoprovocation threshold, and symptom scores.²² The 2 major limitations of this study were that it was likely underpowered to find a difference in the pre-defined outcomes and that after the first year of treatment, there was no longer a placebo group with which to compare further results. The lack of efficacy noted and lack of placebo with which to compare the results lead to a study conclusion that does not support the use of dog immunotherapy.

Notably, the parallel cat immunotherapy arm showed a statistically and clinically significant difference in specific allergen and nonspecific histamine bronchoprovocation. Speculation continued that these findings most likely resulted from potency differences between the cat and dog extracts. The lower local and systemic side effect profile in the dog group also coincided with this theory, but similar immunologic changes in IgE and IgG levels in the dog and cat groups suggest otherwise.

Further Dog Immunotherapy Clinical Studies

Subsequent studies evaluated different hypotheses of why dog immunotherapy did not exhibit robustly successful results. Previously it had been proposed that only the late response is improved with therapy; however, a 1988 study showed that after immunotherapy, 3 of the 5 patients experienced complete inhibition of their immediate response and 1 patient with the dual response experienced ablation of early and late responses.²³ In 1989, another Danish study aimed to evaluate the effects of dog or cat immunotherapy in addition to maximal medical therapy alone (because it was deemed unethical to stop all other treatments) in 27 children with asthma. A statistically significant difference was found in an increased tolerance of dog allergen during bronchoprovocation

testing, suggesting the utility of combining immunotherapy with medical therapy to improve outcomes.²⁴

In 1989, a Swedish study aimed to elucidate the effectiveness of dander immunotherapy with another new, more potent extract in patients with continuous dog exposure. After 3 months, skin prick testing and conjunctival challenges were significantly decreased with little further improvement for the duration of the 12 months. These findings confirmed the effectiveness of immunotherapy in patients with continued allergen exposure.²⁵ An additional study in 1992 treated patients with cat and dog allergies with immunotherapy to cat and dog extracts. The investigators theorized that the lack of response to dog immunotherapy in several past studies could be due to concomitant allergens for which they were not receiving therapy. Unfortunately, patients with dog allergy treated with dog and cat allergens had no decrease in bronchial hyperactivity to histamine or dog extract.²⁶

In 2006, Lent et al²⁷ sought to determine the most effective dose of dog immunotherapy by following immunologic markers at differing doses of Can f 1 up to 15 µg, a dose noted to be the most effective dose in an antecedent study involving cat extract. Because conventional dog extract had previously been reported to contain 5 µg/mL of Can f 1, it was determined that only acetone-precipitated (AP) dog extract (reported by the manufacturer to contain up to 165 µg/mL) was potent enough to reach these therapeutic levels. Twenty-seven patients received cluster immunotherapy for 5 weeks, with interest in the outcomes of skin prick test positivity, nasal challenge, serum specific IgE, and IgG4. After immunotherapy, a dose-dependent response was noted in suppression of skin prick testing and an increase in IgG4, but, importantly, there was no change in symptoms scores after nasal challenge. The investigators concluded that the dog immunotherapy is “likely effective” because many of the same immunologic parameters were similar as the antecedent Fe d 1 dosing trial (which did show clinical efficacy).

The validity of following immunologic parameters, specifically the decrease in IgE or increase in IgG4 alone, as a surrogate for clinical efficacy was questioned in several earlier studies because changes in immunologic markers did not correspond to symptoms or provocative testing results.^{17,19–22,24,25} A subsequent study in 2013 noted similar IgG4 levels in patients sensitized to dog and in patients asymptomatic to dog exposure. This trial provided further evidence to question relying on immunologic parameters alone as a surrogate marker for clinical efficacy.²⁸

The published trial data do support safety of subcutaneous dog immunotherapy. Overall, the systemic reaction rate reported for these dog immunotherapy trials was 0% to 0.3% in subjects receiving dog extracts.^{14–16,19,20,23–25,27}

Early Dog Allergens and Extracts

After dog immunotherapy gained awareness in the 1960s, efforts began to understand the complex antigenic and allergenic profile of dog extracts. As early as 1971, reports noted that the potency of different extracts could vary by up to 1,000-fold and that different breeds appeared to have a different profile of allergens.²⁹ Dog serum albumin (DSA) was identified in 1973 as an important allergen, but it also was noted that there were likely other important allergens. As expected, albumin levels were confirmed to be much higher in epithelial extracts compared with hair extracts.³⁰ Two years later, Ohman et al³¹ explained the frequent finding of cat co-sensitization in patients allergic to dog using IgE inhibition studies that showed common allergens between dogs and cats.

In 1977, Blands et al³² characterized the extracts of 6 different dog breeds and found 21 distinct antigens. DSA and antigen (Ag) 13 were major allergens with IgE binding in more than 50% of symptomatic patients, and there were 6 minor allergens. Significant

cross-reactive allergens with cat were noted, but no breed-specific allergens were identified. Because dogs phenotypically vary wildly, it was still assumed that different breeds would have unique antigens. In 1980, a study into breed specifics noted that dander was more allergenic than serum for all breeds. This study concluded that the variations in skin testing among breeds could be due to qualitative or more likely quantitative differences in extracts because no breed-specific allergens were identified.³³

In 1983, Uhlin et al³⁴ characterized a poodle and Alsatian dandruff extract, noting 29 different antigens and 24 separate allergens. These 2 breeds were chosen because poodle appeared to have higher dander allergen levels, whereas Alsations had higher serum allergen levels. When combined, these 2 breeds would theoretically produce a more balanced and potent extract. In 1985, data analysis of the landmark clinical study noted 16 total antigens in their dog extract being used for treatment. IgE was noted to bind to up to 5 different allergens per patient. IgE also was discovered to bind most often to Ag 2 (DSA), Ag 6, and Ag 13.³⁵

In 1986, the International Union of Immunological Societies sought to standardize the allergen nomenclature system. Highly purified allergens would be designated using the first 3 letters in the genus name, followed by the first letter in the species name in italics, followed by a roman numeral. This numeral was intended to represent the chronologic order in which the molecules were identified with the following 2 major exceptions. The major allergen would normally be assigned “I” regardless of its order of identification and structurally homologous allergens from different species would be given the same number (eg, *Der p* I and *Der f* I).³⁶ This intervention was not immediately universally accepted but was pivotal to ameliorating the confusion about allergens and eliminating redundancy of research. In 1994, the italics were deemed unnecessary and the roman numerals were switched to Arabic numerals, producing the current format.

In 1988, Lindgren et al³⁷ attempted to equalize the different extracts of 8 breeds of dogs in terms of crude protein content to correct for quantitative differences caused by serum allergens and to discover any qualitative differences. With this correction, the study did notice that some allergens were found only in certain breeds and that DSA appeared to represent only a weaker allergen to which only 18% of patients allergic to dog showed skin test positivity. It also noted that approximately 15% of patients showed a significant difference in skin test results based on specific breed, but these differences did not correlate to symptom scores. These differences do not appear to have been pursued further. In 1989, Ford et al³⁸ performed a similar study using sera from British and Finnish patients allergic to dog and found no difference in allergen binding based on the geographic location of the patients. Twenty-one separate allergens were identified, notably Ag 8, which was found primarily in dander and bound IgE in 63% of patients. Ag 8 was in fact the same allergen as the previously named Ag 13, providing another example of the confusion among antigens and the value of adhering to the standardized nomenclature.

Extract Research after Nomenclature Standardization

In 1991, Schou et al³⁹ sought to further characterize Ag 8/13 and named it Can f 1 according to the new nomenclature system. Can f 1 was described as having a molecular weight of 22 to 25 kDa. In the study, 24 of 26 patients (92%) with dog allergy had a positive skin prick reaction to purified Can f 1, but only 16 of 23 patients (70%) demonstrated specific IgE by immunoblotting, and Can f 1 accounted for only 56.5% of IgE binding in pooled sera from patients allergic to dog. These data confirm the importance of Can f 1 but also indicate the role of other allergens. Another study by Ford and Kemeny⁴⁰ in 1992 noted a dander-specific allergen at 21 kDa, naming it Ag X initially but then noting the similarity in size and

binding (78% total) to the Ag 8 of Lindgren et al and the Ag 13 of Blands et al. It was agreed that the allergen was likely the same as the previously named Can f 1.

Once Can f 1 was determined to be an important dog allergen, efforts began to determine its best natural source to improve the potency of the extracts. Can f 1 levels in 4 commercially available extract sources (dander, hair and skin scrapings, skin scrapings, and whole skin) were compared in 1994 using different in vivo and in vitro testing techniques. It was discovered that dander was the best source. Subsequent evaluation showed that Can f 1 is a lipocalin that is produced on tongue epithelia, explaining why Can f 1 is found in the skin and saliva⁴¹ but not in the urine or feces. This testing showed that Can f 1 is typically accompanied by a distinct 19-kDa lipocalin named Can f 2. The source of Can f 2 is the parotid gland and the highest content is in extracts made from dander.⁴²

A 1991 article investigating Can f 1 also mentioned dog allergen 2 (Can f 2), noting IgE binding to this allergen in 34% of patients with symptoms to dog exposure. Can f 2 was noted to be positive only in patients also positive to Can f 1, but not all patients positive to Can f 1 were sensitized to this molecule. However, it was noted that Can f 1 and Can f 2 were found in all commercial cat extracts, suggesting contamination or cross-reactivity.⁴³ The structure of Can f 2 was ultimately revealed by crystallography in 2010. The structure is similar to Fe d 4, likely explaining some of this observation.⁴⁴

In 1994, Spitzauer et al⁴⁵ continued investigating DSA, the 66- to 69-kDa allergen with approximately 35% IgE binding (subsequently named Can f 3), and its cross-reactivity with other mammalian albumins. This investigation was conducted by producing fragments of recombinant albumin. Because mammalian albumins are similar, Fe d 2 and Can f 3 exhibited cross-reactivity, further explaining cat co-sensitization in individuals allergic to dog.

Variability of Dog Extracts

To complicate matters, a 1996 report noted that the major dust mite allergens, Der p 1 and Der p 2, were detectable in all commercial dog extracts at a median concentration of 5.8 ng/mL. This level could explain skin test positivity to dogs in the absence of allergic sensitivity to dogs. Can f 1 levels in these extracts ranged from 3.8 to 170 µg/mL. This study illuminated the possibility of false positives with skin prick testing for dog allergy.⁴⁶

In 2001, Meiser and Nelson⁴⁷ compared the newest AP dog extract to the traditional aqueous dog extract in 123 consecutive patients receiving skin testing and noted that 59 of 123 patients (48%) tested positive to AP dog. Only 35 of 123 patients (28%) tested positive to conventional dog extracts. All patients who tested positive to conventional extracts also were positive to AP dog, with a significantly larger mean wheal diameter. These data perpetuated questions about false negatives because 24 of 59 patients (41%) who tested positive to AP dog tested negative to conventional dog extracts.

Stability of Dog Extracts

A study by Cain et al⁴⁸ in 1998 showed that Can f 1 was relatively thermostable, with 50% of the allergen remaining after 60 minutes

of 140°C dry heat. In 2009, the stability and compatibility of AP and conventional dog extracts were investigated in different time, temperature, and mixing conditions.⁴⁹ Can f 1 levels remained at 94% to 119% and Can f 3 retained 79% to 94% potency for 3 days when kept between 21°C and 45°C, showing standard shipping methods without refrigeration are acceptable for extracts. These extracts also were mixed with molds and insects (cockroach and fire ant) and refrigerated for 15 months. As expected, the extracts with 50% glycerin retained their potency, whereas the extracts with no more than 10% glycerin showed significant degradation. The effects of combining dog extracts with cat dander, pollens, or dust mite was not determined.

Newest Identified Dog Allergens

A plethora of new information regarding dog allergens has been published in the past 5 years (Table 1). In 2009, prostatic kallikrein, a 28-kDa protein, was identified (and named Can f 5) as a major dog allergen because it bound IgE in 70% of patients with allergy. As a prostatic protein, it is found only in male dog urine and exhibits cross-reactivity to human prostate-specific antigen, providing a potential explanation for the development of semen hypersensitivity from dog allergen exposure. Interestingly, 38% of patients reacted only to Can f 5 and not to Can f 1, 2, or 3, suggesting that some individuals are selectively allergic to male dogs.⁵⁰ In 2010, Can f 4, an 18-kDa protein, was characterized as a lipocalin and it was reported to bind 35% of patients' IgE. As a lipocalin, it was found to be cross-reactive with cow dander.⁵¹ In 2012, Can f 6 also was more fully characterized as a 20-kDa lipocalin reported to bind IgE in 35% of patients with dog allergy. As another lipocalin, it was found to be cross-reactive to cat and horse.⁵² In 2013, dog saliva was reported to contain Can f 1, 2, 4, and 6 (all known dog lipocalin allergens), suggesting its potential role for use in extract manufacturing.⁵³

"Major" allergen studies have their own inherent errors because allergens were initially described as major if they bound IgE in more than 90% of patients. However, this number was subsequently decreased to more than 50% because very few allergens qualified. Also, testing methods to prove IgE binding vary among studies and sample size has varied greatly in different studies, with a smaller sample inherently creating less precise rates. Thus, it is clear that there are several important dog allergens, but no dominant allergen. Ultimately, the only "major" allergen is the one to which a particular patient is sensitized!

Current Prescribing Trends

A nationwide survey in 2006 showed the perspective of how allergists across the United States were integrating these conflicting and confusing data and using them in daily practice. Other types of physicians prescribing immunotherapy were excluded. Responses showed the maintenance weight-to-volume ratio varied widely from 1:10 to 1:1,000, with the most common concentration being 1:100 (24.3%). Despite the paucity of clinical evidence, most allergists believe that dog immunotherapy will provide at least some

Table 1
Described allergens of *Canis familiaris*

Allergen (previous names)	Molecular weight (kDa)	Biologic activity	Sensitization rates (%)	Major cross-reactive allergens
Can f 1 (Ag 13, Ag 8, Ag X)	23–25	Lipocalin	50–70	Unknown
Can f 2	19	Lipocalin	30	Fe d 4
Can f 3 (dog serum albumin, Ag 2)	66–69	Albumin	35	Fe d 2
Can f 4	16–23	Lipocalin	35	Bos d 2
Can f 5	28	Prostatic kallikrein	70	Human prostate-specific antigen
Can f 6	20	Lipocalin	35	Fe d 4, Equ c 1

Abbreviation: Ag, antigen.

relief from allergic rhinitis (91.9%) and asthma (89.6%), and 86% would be willing to offer immunotherapy to treat occupational allergy. This rate decreases to 81% if the patient is unable to remove the dog for purely emotional reasons.⁵⁴ These results show a more optimistic view of dog immunotherapy compared with 1987 when only 65% of board-certified allergists in private practice perceived improvement in allergic rhinitis or asthma symptoms with cat or dog immunotherapy.⁵⁵

The Future of Dog Allergy

This new information about the specific allergens of dogs could help clarify the diagnosis and treatment of dog allergy. Component testing using antibody-based measurement of patients with dog allergy will help characterize each patient's particular sensitization pattern (Can f 1 to 6) to dogs. With this information, it will be possible to help deduce primary dog sensitization vs symptoms that are due to cross-reactivity from another animal allergy. Recombinant allergens have been manufactured for decades. Fragments of Can f 3 were the first to be produced in a recombinant form in 1993⁴⁵ and fully produced as a recombinant protein in 2000.⁵⁶ Can f 1 and Can f 2 were cloned in 1997⁵⁷ and were investigated for diagnostic purposes.⁵⁸ In 2014, a multimeric recombinant allergen that contained Can f 1, 2, 4, and 6 was created for diagnostic and therapeutic purposes.⁵⁹ Unfortunately, recombinant allergens often lose sensitivity compared with the natural form, suggesting they might not be ready for clinical application, and with an unclear timeline for their refinement, should not discourage the use of current extracts. Research into the optimal organism in which to produce recombinant proteins continues. In the future, more potent and patient specific extracts could be developed from a mixture of organic sources (saliva, urine, and dander) and hybrid extracts with natural and recombinant allergens could be produced for use in skin testing and immunotherapy. Component testing and patient-specific hybridized extracts could identify and provide the best therapeutic match to the patient's particular pattern of sensitivities and therefore offer better chances for effective therapy.

Conclusion

Studies investigating dog allergen immunotherapy during the past 40 years have failed to demonstrate any clearly reproducible clinical evidence to confirm its effectiveness in ameliorating symptoms of rhinitis and asthma. Despite these data, US allergists typically include dog extracts in aeroallergen immunotherapy for sensitized patients. Future research is necessary to produce more potent and complex extracts that could be tailored to an individual patient's pattern of dog sensitization, but the first step would be to produce a standardized extract for clinical use. Thereafter, additional trials could be designed to evaluate the efficacy of dog allergen immunotherapy in improving clinical end points for patients rather than merely changing their immunologic parameters. Properly powered studies with consistent allergen extracts also would facilitate identification of the optimal dose required to produce these effects, if one exists. Until that time, the current recommendation on immunotherapy dosing for nonstandardized dog extracts is 15 µg of Can f 1 per dose; with the current extracts available in the United States, this can be obtained only with a 0.5-mL dose using full-strength (1:100 w/v) AP dog extract.⁶⁰

References

[1] Custovic A, Green R, Taggart SC, et al. Domestic allergens in public places. II: dog (Can f 1) and cockroach (Bla g 2) allergens in dust and mite, cat, dog and cockroach allergens in the air in public buildings. *Clin Exp Allergy*. 1996;26:1246–1252.

[2] Custovic A, Simpson BM, Simpson A, et al. Current mite, cat, and dog allergen exposure, pet ownership, and sensitization to inhalant allergens in adults. *J Allergy Clin Immunol*. 2003;111:402–407.

[3] Arshad SH, Tariq SM, Matthews S, Hakim E. Sensitization to common allergens and its association with allergic disorders at age 4 years: a whole population birth cohort study. *Pediatrics*. 2001;108:E33.

[4] Ownby DR, Johnson CC, Peterson EL. Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. *JAMA*. 2002;288:963–972.

[5] Park HJ, Lee JH, Park KH, et al. A nationwide survey of inhalant allergens sensitization and levels of indoor major allergens in Korea. *Allergy Asthma Immunol Res*. 2014;6:222–227.

[6] Hodson T, Custovic A, Simpson A, Chapman M, Woodcock A, Green R. Washing the dog reduces dog allergen levels, but the dog needs to be washed twice a week. *J Allergy Clin Immunol*. 1999;103:581–585.

[7] Popplewell EJ, Innes VA, Lloyd-Hughes S, et al. The effect of high-efficiency and standard vacuum-cleaners on mite, cat and dog allergen levels and clinical progress. *Pediatr Allergy Immunol*. 2000;11:142–148.

[8] Green R, Simpson A, Custovic A, Woodcock A. Vacuum cleaners and airborne dog allergen. *Allergy*. 1999;54:403–405.

[9] Vredegoor DW, Willemsse T, Chapman MD, Heederik DJ, Krop EJ. Can f 1 levels in hair and homes of different dog breeds: lack of evidence to describe any dog breed as hypoallergenic. *J Allergy Clin Immunol*. 2012;130:904–909.e7.

[10] Ramadour M, Guetat M, Guetat J, El Biase M, Magnan A, Vervloet D. Dog factor differences in Can f 1 allergen production. *Allergy*. 2005;60:1060–1064.

[11] Gould M. Treatment with cat and dog epithelial suspensions. Presented at the American Academy of Allergy Meeting; 1963.

[12] Tuft L, Torsney PJ. Experience in the hyposensitization treatment of animal dander allergic patients with alum-precipitated pyridine extracts. *Am J Med Sci*. 1967;253:19–22.

[13] Scherr M. Letters of the International Correspondence of the Society of Allergists. 1967:92–94.

[14] Brown FR, Wolfe HI. Observations on animal dander hyposensitization. *Ann Allergy*. 1968;26:305–308.

[15] Tuft L, Torsney PJ, Kreithen H, Heck VM. Hyposensitization therapy with alum-precipitated pyridine extracts in animal dander sensitive patients. *Ann Allergy*. 1976;36:165–173.

[16] Valovirta E, Koivikko A, Vanto T, Viander M, Ingeman L. Immunotherapy in allergy to dog: a double-blind clinical study. *Ann Allergy*. 1984;53:85–88.

[17] Valovirta E, Viander M, Koivikko A, Vanto T, Ingeman L. Immunotherapy in allergy to dog. Immunologic and clinical findings of a double-blind study. *Ann Allergy*. 1986;57:173–179.

[18] Sundin B, Lilja G, Graff-Lonnevig V, et al. Immunotherapy with partially purified and standardized animal dander extracts. I. Clinical results from a double-blind study on patients with animal dander asthma. *J Allergy Clin Immunol*. 1986;77:478–487.

[19] Hedlin G, Graff-Lonnevig V, Heilborn H, et al. Immunotherapy with cat- and dog-dander extracts. II. In vivo and in vitro immunologic effects observed in a 1-year double-blind placebo study. *J Allergy Clin Immunol*. 1986;77:488–496.

[20] Lilja G, Sundin B, Graff-Lonnevig V, et al. Immunotherapy with cat- and dog-dander extracts. IV. Effects of 2 years of treatment. *J Allergy Clin Immunol*. 1989;83:37–44.

[21] Hedlin G, Graff-Lonnevig V, Heilborn H, et al. Immunotherapy with cat- and dog-dander extracts. V. Effects of 3 years of treatment. *J Allergy Clin Immunol*. 1991;87:955–964.

[22] Hedlin G, Heilborn H, Lilja G, et al. Long-term follow-up of patients treated with a three-year course of cat or dog immunotherapy. *J Allergy Clin Immunol*. 1995;96:879–885.

[23] Rohatgi N, Dunn K, Chai H. Cat- or dog-induced immediate and late asthmatic responses before and after immunotherapy. *J Allergy Clin Immunol*. 1988;82:389–397.

[24] Bertelsen A, Andersen JB, Christensen J, Ingemann L, Kristensen T, Ostergaard PA. Immunotherapy with dog and cat extracts in children. *Allergy*. 1989;44:330–335.

[25] Bucur J, Dreborg S, Einarsson R, Ljungstedt-Pahlman I, Nilsson JE, Persson G. Immunotherapy with dog and cat allergen preparations in dog-sensitive and cat-sensitive asthmatics. *Ann Allergy*. 1989;62:355–361.

[26] Haugaard L, Dahl R. Immunotherapy in patients allergic to cat and dog dander. I. Clinical results. *Allergy*. 1992;47:249–254.

[27] Lent AM, Harbeck R, Strand M, et al. Immunologic response to administration of standardized dog allergen extract at differing doses. *J Allergy Clin Immunol*. 2006;118:1249–1256.

[28] Burnett M, Wegienka G, Havstad S, et al. Relationship of dog- and cat-specific IgE and IgG4 levels to allergic symptoms on pet exposure. *J Allergy Clin Immunol Pract*. 2013;1:350–353.

[29] Varga JM, Ceska M. Characterization of allergen extracts by polyacrylamide gel isoelectrofocusing and radioimmunosorbent allergen assay. II. Dog and cat allergens. *Int Arch Allergy Appl Immunol*. 1972;42:438–453.

[30] Yman L, Brandt R, Ponterius G. Serum albumin—an important allergen in dog epithelia extracts. *Int Arch Allergy Appl Immunol*. 1973;44:358–368.

[31] Ohman JL Jr, Bloch KJ, Kendall S, Lowell FC. Allergens of mammalian origin. IV. Evidence for common allergens in cat and dog serum. *J Allergy Clin Immunol*. 1976;57:560–568.

- [32] Blands J, Lowenstein H, Weeke B. Characterization of extract of dog hair and dandruff from six different dog breeds by quantitative immunoelectrophoresis. Identification of allergens by crossed radioimmunoelectrophoresis (CRIE). *Acta Allergol.* 1977;32:147–169.
- [33] Moore BS, Hyde JS. Breed-specific dog hypersensitivity in humans. *J Allergy Clin Immunol.* 1980;66:198–203.
- [34] Uhlin T, Reuterby J, Einarsson R. Antigenic/allergenic composition of poodle/Alsatian dandruff extract. *Allergy.* 1984;39:125–133.
- [35] Lowenstein H, Graff-Lonnevig V, Hedlin G, et al. Immunotherapy with cat and dog-dander extracts. III. Allergen-specific immunoglobulin responses in a 1-year double-blind placebo study. *J Allergy Clin Immunol.* 1986;77:497–505.
- [36] Marsh DG, Goodfriend L, King TP, Lowenstein H, Platts-Mills TA. Allergen nomenclature. *Bull World Health Organ.* 1986;64:767–774.
- [37] Lindgren S, Belin L, Dreborg S, Einarsson R, Pählman I. Breed-specific dog-dandruff allergens. *J Allergy Clin Immunol.* 1988;82:196–204.
- [38] Ford AW, Alterman L, Kemeny DM. The allergens of dog. I. Identification using crossed radio-immunoelectrophoresis. *Clin Exp Allergy.* 1989;19:183–190.
- [39] Schou C, Svendsen UG, Lowenstein H. Purification and characterization of the major dog allergen, Can f 1. *Clin Exp Allergy.* 1991;21:321–328.
- [40] Ford AW, Kemeny DM. The allergens of dog. II. Identification and partial purification of a major dander allergen. *Clin Exp Allergy.* 1992;22:793–803.
- [41] Martínez A, Martínez J, Sanz ML, Bartolomé B, Palacios R. Dander is the best epithelial source for dog allergenic extract preparations. *Allergy.* 1994;49:664–667.
- [42] Spitzauer S, Schweiger C, Anrather J, et al. Characterisation of dog allergens by means of immunoblotting. *Int Arch Allergy Immunol.* 1993;100:60–67.
- [43] de Groot H, Goei KG, van Swieten P, et al. Affinity purification of a major and a minor allergen from dog extract: serologic activity of affinity-purified Can f 1 and of Can f 1–depleted extract. *J Allergy Clin Immunol.* 1991;87:1056–1065.
- [44] Madhurantakam C, Nilsson OB, Uchtenhagen H, et al. Crystal structure of the dog lipocalin allergen Can f 2: implications for cross-reactivity to the cat allergen Fe d 4. *J Mol Biol.* 2010;401:68–83.
- [45] Spitzauer S, Schweiger C, Sperr WR, et al. Molecular characterization of dog albumin as a cross-reactive allergen. *J Allergy Clin Immunol.* 1994;93:614–627.
- [46] van der Veen MJ, Mulder M, Witteman AM, et al. False-positive skin prick test responses to commercially available dog dander extracts caused by contamination with house dust mite (*Dermatophagoides pteronyssinus*) allergens. *J Allergy Clin Immunol.* 1996;98:1028–1034.
- [47] Meiser JB, Nelson HS. Comparing conventional and acetone-precipitated dog allergen extract skin testing. *J Allergy Clin Immunol.* 2001;107:744–745.
- [48] Cain G, Elderfield AJ, Green R, et al. The effect of dry heat on mite, cat, and dog allergens. *Allergy.* 1998;53:1213–1215.
- [49] Grier TJ, LeFevre DM, Duncan EA, Esch RE. Stability and mixing compatibility of dog epithelia and dog dander allergens. *Ann Allergy Asthma Immunol.* 2009;103:411–417.
- [50] Mattsson L, Lundgren T, Everberg H, Larsson H, Lidholm J. Prostatic kallikrein: a new major dog allergen. *J Allergy Clin Immunol.* 2009;123:362–368.
- [51] Mattsson L, Lundgren T, Olsson P, Sundberg M, Lidholm J. Molecular and immunological characterization of Can f 4: a dog dander allergen cross-reactive with a 23 kDa odorant-binding protein in cow dander. *Clin Exp Allergy.* 2010;40:1276–1287.
- [52] Nilsson OB, Binnmyr J, Zoltowska A, Saarne T, van Hage M, Grönlund H. Characterization of the dog lipocalin allergen Can f 6: the role in cross-reactivity with cat and horse. *Allergy.* 2012;67:751–757.
- [53] Polovic N, Wadén K, Binnmyr J, et al. Dog saliva—an important source of dog allergens. *Allergy.* 2013;68:585–592.
- [54] Coop C, Johnson T, Hagan L, Steven S. Dog immunotherapy practices among allergists. *Internet J Asthma Allergy Immunol.* 2006;5: number 2.
- [55] Grammer LC, Shaughnessy MA, Shaughnessy JJ, Patterson R. Practices in cat and dog immunotherapy among allergists. *N Engl J Allergy Proc.* 1987;8:253–258.
- [56] Pandjaitan B, Swoboda I, Brandesky-Pichler F, Rumpold H, Valenta R, Spitzauer S. *Escherichia coli* expression and purification of recombinant dog albumin, a cross-reactive animal allergen. *J Allergy Clin Immunol.* 2000;105:279–285.
- [57] Konieczny A, Morgenstern JP, Bizinkauskas CB, et al. The major dog allergens, Can f 1 and Can f 2, are salivary lipocalin proteins: cloning and immunological characterization of the recombinant forms. *Immunology.* 1997;92:577–586.
- [58] Saarelainen S, Taivainen A, Rytönen-Nissinen M, et al. Assessment of recombinant dog allergens Can f 1 and Can f 2 for the diagnosis of dog allergy. *Clin Exp Allergy.* 2004;34:1576–1582.
- [59] Nilsson OB, Neimert-Andersson T, Bronge M, et al. Designing a multimer allergen for diagnosis and immunotherapy of dog allergic patients. *PLOS One.* 2014;9:e111041.
- [60] Cox L, Nelson H, Lockey R, et al. Allergen immunotherapy: a practice parameter third update. *J Allergy Clin Immunol.* 2011;127(suppl):1–55.