

Proof of concept of the preventive efficacy of high-dose recombinant mono-allergen immunotherapy in atopic dogs sensitized to the *Dermatophagoides farinae* allergen Der f 2

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Background – Allergen immunotherapy is currently the only intervention proposed to specifically prevent clinical flares after allergen challenges. The low molecular weight Der f 2 (Df2) is a major allergen in Japanese dogs sensitized to *Dermatophagoides farinae* house dust mites.

Objectives – Pilot, blinded, placebo-controlled experiment testing the efficacy of subcutaneous immunotherapy (SCIT) with high doses of recombinant Df2 conjugated to the maltotriose pullulan (rDf2-P).

Methods – Eight Maltese beagle atopic dogs were sensitized to rDf2 then randomized to SCIT with rDf2-P (six dogs) or placebo (two). The immunotherapy consisted of six weekly injections of increasing doses (0.1–10.0 µg) of rDf2-P followed by four monthly injections of 10 µg of this allergen. Epicutaneous rDf2 challenges, rDf2-specific IgE serology and intradermal reactivity, as well as serum cytokine level measurements, were performed throughout the study.

Results – Subcutaneous injections of placebo did not alter the cutaneous reactivity after rDf2 challenge, while that of the dogs treated with rDf2-P SCIT disappeared in five of six dogs (83%) and was reduced in one of six (17%). During SCIT maintenance, skin lesion scores were significantly lower in dogs receiving SCIT compared to those treated with placebo. This clinical improvement was accompanied by a concurrent, yet not significant, decrease in rDf2-specific IgE serology and immediate intradermal reactivity. Cytokine serum levels were inconclusive. There were no adverse events seen with rDf2-P SCIT.

Conclusions and clinical importance – The new mono-allergen SCIT appears safe and effective for reducing skin lesions after allergen challenges; it deserves further testing in dogs with spontaneous atopic dermatitis.

Introduction

Allergen immunotherapy (AIT), defined as the practice of administering escalating amounts of an allergen extract to an allergic subject, is one of the rare interventions that can both alter the immune response to, and prevent relapses of clinical signs after contact with, offending allergens. Allergen immunotherapy thus has the potential to lead to long-term control of allergic diseases.¹ The current consensus on the mechanism of action of AIT proposes a combination of immune changes.² These include: an early desensitization of mast cells and basophils, a reduction of type 2 helper T cells (Th2 cells) and an induction of interleukin-10 (IL-10)-secreting inducible regulatory T and B cells. A downregulation of IgE, a stimulation of production of tolerogenic or blocking allergen-

specific IgG4, and, finally, an induction of innate lymphoid cells also are considered to contribute to the mode of action of this intervention.²

An important caveat of AIT in companion animals is the lack of standardization of extracts used for this intervention; this likely leads to variable concentrations, and likely insufficient doses, of major allergens in the formulations administered. Furthermore, AIT protocols are not optimized with the dual aim of maximizing efficacy while maintaining lower adverse effects in atopic dogs. As a means to alleviate this important limitation, AIT standardized with known quantities of purified or recombinant major allergens (i.e. those to which more than 50% of patients allergic to the parent extract react) would bring distinct molecular, immunological and biological attributes. Moreover, the addition of adjuvants activating innate immune responses and inducing type-1 helper T cells (Th1) and Tregs could bring synergism to high-dose recombinant AIT; altogether, such combination would likely lead to immunotherapy protocols with increased potential for allergen tolerization.²

That 75% of Japanese atopic dogs sensitized to the *Dermatophagoides farinae* house dust mite (HDM) have

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IgE against Der f 2,³ provides a rationale to develop and test adjuvanted AIT against this single allergen.

In this proof of concept trial, we tested if subcutaneous immunotherapy (SCIT) with recombinant Der f 2 (rDf2) adjuvanted with the maltotriose polymer pullulan (Allermune HDM, Nippon Zenyaku Kogyo – Zenoaq; Fukushima, Japan) could prevent allergen induced skin lesions and alter specific hypersensitivity responses in experimentally sensitized atopic dogs.

Materials and methods

All animal experiments performed in this study had been preapproved by the institution's Animal Care and Use Committee.

Animals

For this study, we selected eight Maltese-beagle atopic (MBA)-crossbred dogs.^{4,5} There were six males and two females; their age ranged from 2 to 11 years (average: 4 years). Two dogs per group would be needed for this study to have an 80% power to detect a 50% reduction in skin lesion scores (SLS) between placebo and SCIT interventions with a standard deviation of 20% (one-sided analyses with SCIT < placebo scores, $P = 5\%$; www.powersamplesize.com). To increase the potential for detection of a positive clinical outcome and avoid study underpowering, we decided to treat three times more dogs with SCIT than with placebo.

Sensitization to Der f 2

We pretreated all dogs with oral hydroxyzine at 2 mg/kg twice daily for 3 days before each sensitizing injection.⁶ Dogs were then injected three times, 2 weeks apart, with 200 µg of baculovirus-expressed rDf2 produced in silkworms (Nippon Zenyaku Kogyo – Zenoaq); the first injection was intraperitoneal and the two others subcutaneous. One month and then 2 months later, 500 µg of rDf2 was applied epicutaneously to the axilla after ten tape strips on three consecutive days. Two similar challenges were done 1 week before starting AIT. During this sensitization phase, IDTs and allergen specific IgE serology were done periodically to monitor the rise of rDf2-specific hypersensitivity.

Der f 2 subcutaneous immunotherapy

We randomized eight MBA dogs by computer to receive SCIT or placebo. In the induction (I) phase of SCIT, six rDf2-sensitized MBA dogs were treated, once weekly, with six subcutaneous injections of 1 mL of increasing doses (I-1 to I-6, 0.1–10.0 µg) of rDf2-pullulan (rDf2-P) (Figure 1). In the maintenance (M) phase starting 1 month later, these six dogs received four monthly injections of 10.0 µg of

rDf2-P (M-1 to M-4; Figure 1). We injected the two remaining dogs with 1 mL of saline (placebo) at the same time points.

Epicutaneous allergen challenges

In the 2 weeks preceding the first injection, the 1 month after the last induction (I-6) and the second (M-2) and fourth (M-4) maintenance injections (Figure 1), epicutaneous allergen challenges were performed. At least 24 h before a challenge, hair was clipped on the right axilla. Before allergen application, ten tape strips were done using 2.5 cm wide acetate tape. Allergen challenges consisted of 500 µg of rDf2 in suspension in 0.25 mL of mineral oil. Forty eight hours later the first author, blinded to the intervention, scored erythematous macules, oedema, papules/pustules and excoriations as 0 (absent), 1 (faint, mild) or 2 (strong, severe). The grades for each lesion were added to yield a skin lesion score (SLS). Any SLS ≥ 1 was considered a positive reaction.

Der f 2 specific IgE serum levels

During the sensitization, induction and maintenance phases, blood was serially collected (Figure 1) and serum was isolated for rDf2-specific IgE determination. For this indirect ELISA, plates were first coated with 1 µg rDf2 per well overnight at 4°C. After rinsing with phosphate buffered saline/Tris (PBST), plates were blocked with 10% fetal calf serum (Sigma-Aldrich; St Louis, MO, USA) and 1% sodium azide for one hour at 37°C. After rinsing with PBST, 100 µL of each dog's serum diluted serially (starting at 1:10 and then diluted as needed to achieve plateau reading) were added, in triplicate, and incubated for 1 h at room temperature. After additional washes, 100 µg of biotinylated monoclonal anticanine IgE monoclonal antibody 5.91 was added and incubated for 1 h at room temperature.⁷ The enzyme fluorimetric reaction involved streptavidin-beta-galactosidase (1 h incubation at room temperature) and 4-methylumbelliferyl B-D-galactopyranoside (4MU; 1 h at 37°C). Results were matched to a standard curve using the serum of a dog experimentally sensitized to Df2 and graded at 100 arbitrary units (AU) at 1:50 dilution. The threshold of positivity, calculated previously as the mean + 3 SD of ten normal dogs, was determined to be 50 AU. To minimize interassay variability, we retested all sera at study end, with all blood samples from the same dog run on the same day.

Der f 2 intradermal testing

During the sensitization phase, before the induction and 1 month after the last maintenance injection (M-4) (Figure 1), IDT was done with or without sedation as needed. Injections consisted of a phosphate buffered saline (PBS) negative control, histamine positive control and 2 and 20 µg/mL solutions of rDf2; the latter corresponding to intradermal injections (~0.1 mL) of approximately 200 ng and 2 µg of rDf2. We assessed the immediate IDT reactivity after 20 min by

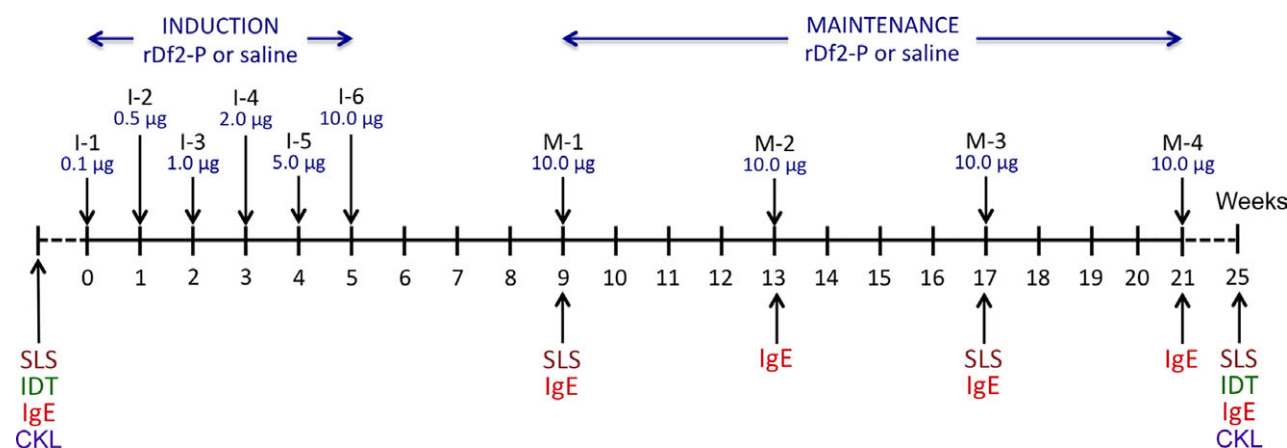


Figure 1. Interventions timeline. CKL serum cytokine levels, I-x induction injection number, IDT intradermal testing, IgE rDf2-specific IgE serology, M-x maintenance injection number, SLS skin lesion score; wks weeks.

calculating a global wheal score (GWS) as reported previously;⁶ GWS = diameter (mm) × erythema (1–3) × firmness (1–3).

Cytokine serum levels

Before commencing treatment and 1 month after the end of maintenance therapy (M-4) (Figure 1), serum was collected for cytokine level measurements. We kept serum frozen until the end of the study when all sera were analysed simultaneously using the Milliplex MAP canine cytokine magnetic bead multiplex assay (Millipore; Billerica, MA, USA). Cytokines of interest were interleukin (IL)-2, -4 and -10 as well as interferon-gamma. The lower limit of detection of the assay was 3.2 pg/mL.

Statistical analyses

For the comparisons of values at the various time points within each treatment group, we used a repeated-measures ANOVA (Friedman test) with post-tests (SLS and IgE) or a paired *t*-test (IDT). For comparisons between groups at each time point, we employed a one-tailed *t*-test due to the unidirectional nature of our hypothesis (SCIT < placebo). We used Prism 5.0 (Graphpad; La Jolla, CA, USA) for statistical analyses and the threshold of significance was $P < 0.05$.

Results

Sensitization to Der f 2

Before sensitization, none of the eight MBA dogs had detectable rDf2-specific serum IgE. At the end of this period all dogs had measurable anti-rDf2 IgE with values ranging from 360 to 5,181 AU. Similarly, at the onset of the sensitization phase, dogs had no visible immediate reactions in IDT with 200 ng and 2 µg of rDf2. At the end of this period, all dogs reacted to both concentrations of this allergen. Finally, none of the eight dogs had any positive reaction (SLS ≥ 1), 48 h after epicutaneous application of rDf2, before being sensitized to this allergen; all dogs exhibited mild reactions to this allergen at the end of this phase.

During this sensitization to rDf2, one dog vomited multiple times after the second intraperitoneal injection, while another dog exhibited transient facial swelling at the same time. Both dogs were treated symptomatically and finished the sensitization without further adverse events.

Subcutaneous immunotherapy with recombinant Der f 2-pullulan

Subcutaneous injections of 0.1–10 µg of rDf2-P did not result in any visible adverse events during this study. Importantly, both dogs that had allergic reactions during the sensitization to rDf2 did not experience any abnormal clinical signs after being injected with rDf2-P.

Epicutaneous allergen challenges

In the placebo group, both dogs continued to exhibit positive cutaneous reactions throughout the study; after the last challenge, the scores of these two dogs were either the same or higher than before treatment. In contrast, all lesional scores decreased after rDf2-P SCIT injections, and the proportion of dogs with positive clinical reactions declined from six of six before treatment (100%) to two of six (33%), 1 month after the last induction phase (I-6) injection. Only one dog (17%) mildly reacted to an allergen challenge 1 month after the end of maintenance (M-4; Figure 2).

Subcutaneous injections of the saline placebo did not result in a significant difference in SLS over time (Friedman test, $P = 0.208$). In contrast, SCIT with rDf2-P resulted in a significant reduction in SLS (Friedman test, $P = 0.003$); values 1 month after the 4th maintenance injection (M-4; approximately 6 months after SCIT onset) were significantly lower than those before treatment (Dunn's post-test, $P < 0.05$).

While SLS were not significantly different between the two treatment groups before and 1 month after the induction phase, they were significantly lower 1 month after M-2 and M-4 SCIT injections compared to placebo ones (*t*-tests, $P = 0.002$ and 0.0005 , respectively).

rDf2 specific IgE serum levels

The rDf2 ELISA was found to have an acceptable reproducibility with an intra-assay CV% of 10.6% and an interassay CV% of 9.9% (data not shown). Altogether, in dogs from both placebo and SCIT groups, rDf2 IgE did not change significantly during the study (Friedman test, $P = 0.500$ and 0.146 , respectively). Serum allergen specific IgE values were not significantly different between the two groups of dogs at any time point (*t*-test, $P > 0.05$). At the end of the study, 1 month after the last maintenance (M-4) injection, rDf2-specific IgE values were lower than at baseline in one of the two dogs treated with placebo and in five of six (83%) dogs treated with SCIT (Figure 3). The median percentage changes of AU during treatment were +79% (placebo) and -66% (SCIT).

Intradermal testing

Results of IDT with the two doses of rDf2 were similar. Within each treatment group, GWS were not significantly different 1 month after the 4th maintenance injection (M-4) compared to baseline (paired *t*-test, $P > 0.05$). At both time points, values were not significantly different between groups (*t*-test, $P > 0.05$). At the end of treatment, GWS had decreased in one of two dogs injected with saline and in four of six treated with rDf2-P (67%) (Figure 3); in placebo and SCIT treated dogs, the median changes from baseline GWS were -6 and -11%, respectively.

Cytokine serum levels

Before and after treatment serum levels of the cytokines relevant to AIT can be found in Table S1. Altogether, few sera had levels of cytokines above the lower limit of detection and levels were variable over time. Importantly, we did not detect any rise in serum IL-10, 1 month after the end of maintenance (M-4) compared to before SCIT.

Discussion

In this experimental proof of concept study, subcutaneous injections of pullulan-adjuvanted rDf2 were able to induce a reduction in the cutaneous reactivity to this allergen. In most dogs this beneficial effect was associated with a concurrent, yet not significant, decrease in allergen specific IgE serology and intradermal reactivity. Results from this pilot study provide a rationale for further testing of this high-dose AIT in atopic dogs naturally sensitized to this allergen.

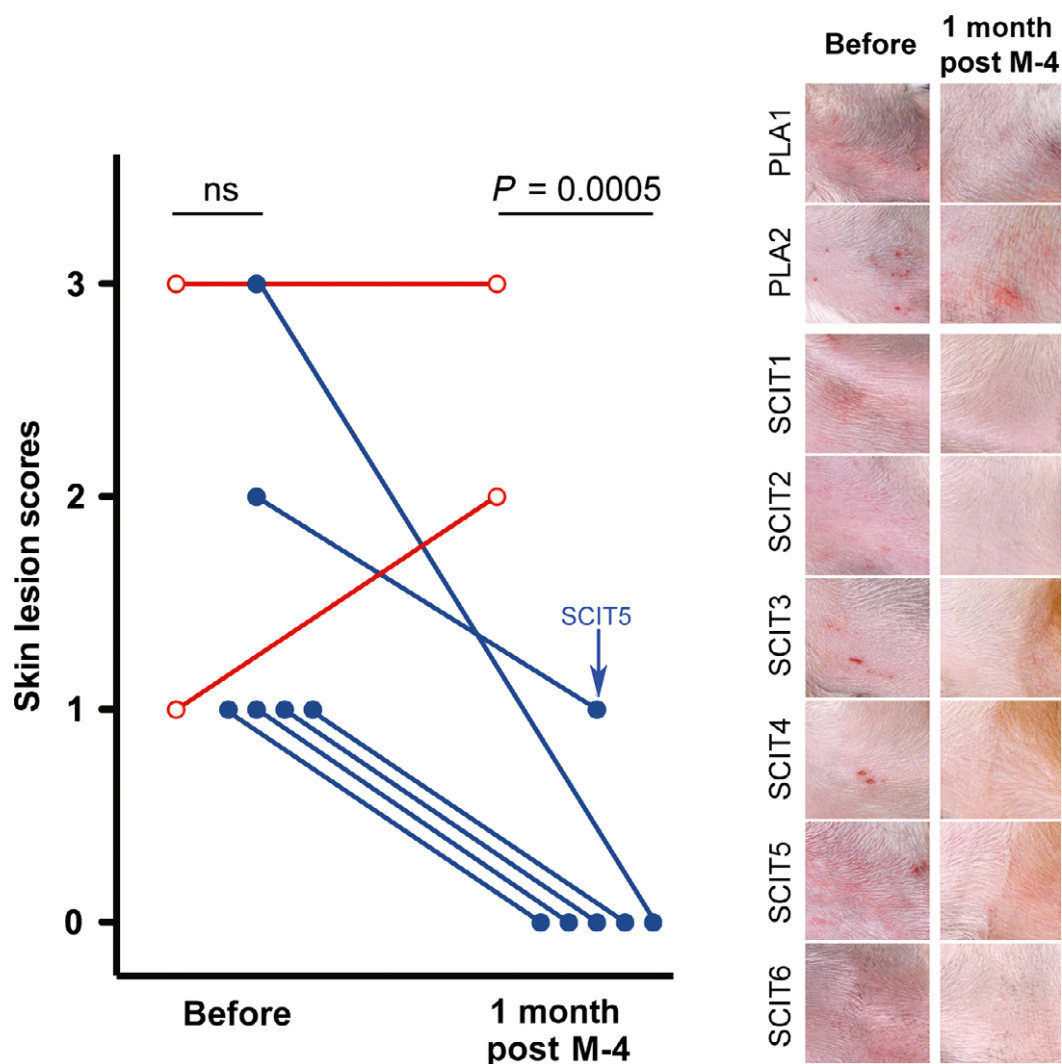


Figure 2. Evolution of skin lesion scores during treatment. ns not significant, SCITx number of the dog. Red = placebo; blue = SCIT.

After rDf2-P SCIT, the SLS after allergen challenges were significantly lower than those after placebo, as early as 1 month after the 2nd monthly maintenance injection (M-2; approximately 4 months after beginning this intervention). Allergen induced cutaneous reactivity disappeared completely in all but one dog treated at trial's end. This high percentage of clinical response to rDf2-P SCIT in our sensitized atopic dogs is comparable to, or better than, that historically seen after traditional⁸ or rush SCIT.⁹ Similarly, the time needed to obtain a positive clinical response with this standardized mono-allergen protocol appeared shorter than in most studies reporting the efficacy of SCIT in dogs with spontaneous atopic dermatitis (AD).^{8,9} Indeed, clinical reactivity after rDf2 allergen challenge was no longer seen in two thirds of our dogs 1 month after the last induction injection, that is approximately 2 months after starting treatment. Such rapid onset of efficacy could be due to the high dose of allergen injected, to the pullulan adjuvant, or to both.

The World Health Organization guidelines define optimal doses of AIT as those that induce benefit in most treated patients without causing adverse effects;¹ the optimal dose for AIT is reported to be between 5 and 20

µg of major allergens from house dust mites, cat dander, pollen or *Hymenoptera* venom.¹ In support of such recommendation, a clinical trial of AIT for human patients with mite allergic asthma showed a dose dependent clinical efficacy.¹⁰ A maximum effect was seen with a formulation containing 7 µg of the major *Dermatophagoides* allergen Der p 1 and there was no added clinical benefit, but a stronger risk of adverse effects, with higher doses of allergens.¹⁰ In another study, AIT with high doses (≥4 µg) of Der p 1 led to more positive changes in relevant immunological parameters than with lower doses of this allergen.¹¹ The SCIT protocol used in our study began with a six dose escalation from 0.1 to 10 µg of rDf2 followed by a maintenance phase of four monthly injections of 10 µg of this allergen. This dose range corresponds to the WHO recommendations for optimal major house dust mite allergens in AIT,¹ and these doses are on par with those leading to maximal clinical efficacy in humans with mite allergic asthma.^{10,11} As a result, the high doses of rDf2 used in this study could be an explanation for the rapid decrease in skin reactions after allergen challenge.

This early clinical benefit could also be due to the unique adjuvant used. Polymers of pullulan, an alpha-

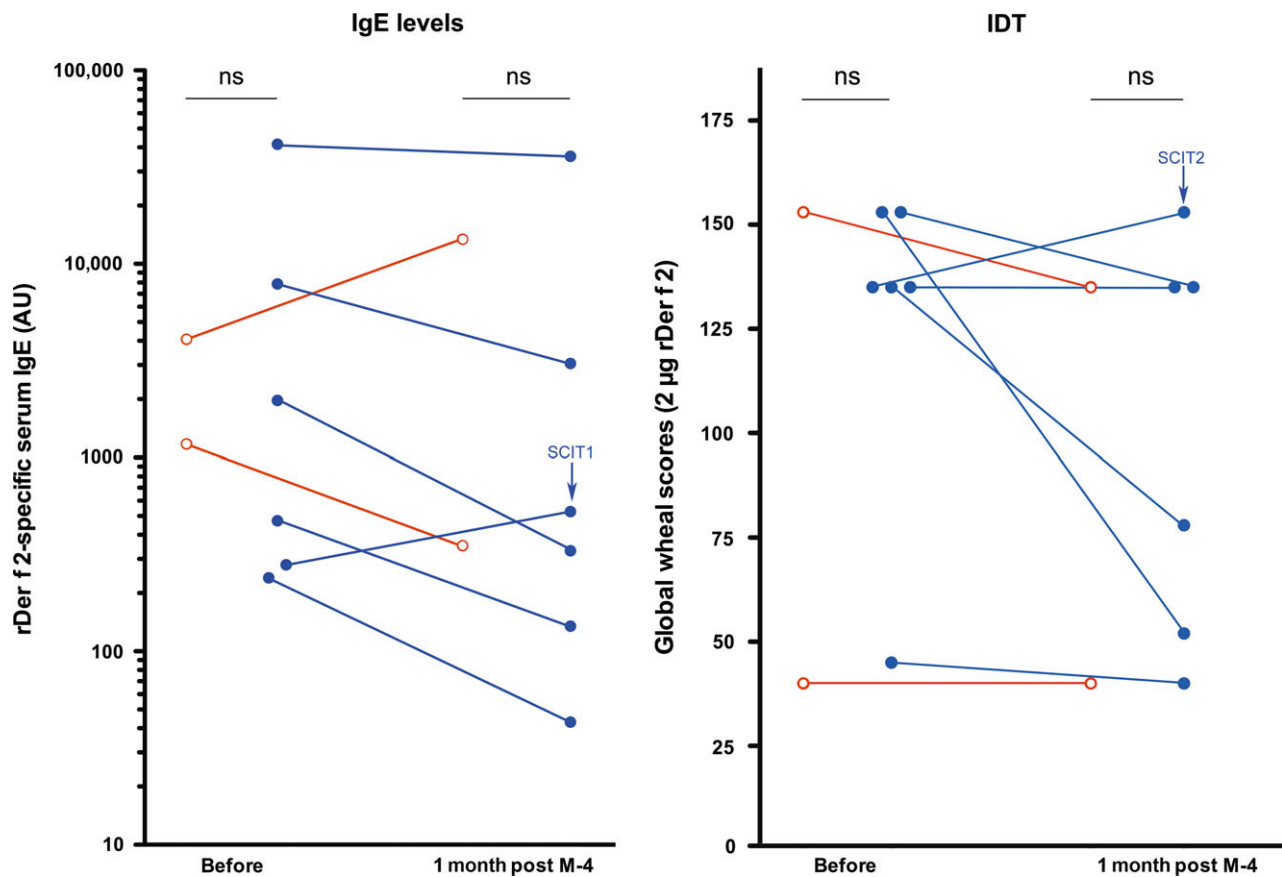


Figure 3. Evolution of allergen-specific IgE serum levels and intradermal reactivity during rDf2-P treatment. ns not significant, SCITx number of the dog. Red = placebo; blue = SCIT.

glucan with a basic unit composed of three alpha-1,4-linked glucose molecules (maltotriose) connected by alpha-1,6-linkages on the terminal glucose, are known not only to enhance the plasma half-life of conjugated molecules, but they can also reduce their IgE recognition and hence their allergenicity.¹² Furthermore, pullulan has been shown to exhibit immunomodulating properties that are desirable in an AIT adjuvant, for example, the promotion of secretion of pro-inflammatory (IL-1-beta, IL-6, IL-8/CXCL8 and TNF-alpha)^{13,14} and type-1 cytokines such as IL-23 and interferon-alpha and -gamma.^{14,15}

In this study, the levels of rDf2-specific IgE were reduced in all but one dog treated with SCIT, but the changes were not significantly different within or between groups. There are only two reports of decreases in allergen specific IgE serum levels in atopic dogs treated with AIT.^{16,17} After 1 year of SCIT, a significant reduction in allergen specific IgE was found only in dogs whose signs were deemed to have responded to this intervention.¹⁶ The magnitude of this change was approximately one half to two thirds average decreases in serum levels depending upon the allergen.¹⁶ After SLIT, mite specific IgE serum titres fluctuated in a zigzag pattern that mirrored the dosing regimen; levels were on average less than 10% of baseline values after only 6 months of treatment.¹⁷ In contrast to these observations, allergen specific IgE serum levels remained unchanged during 7 months of oral house dust mite specific AIT.¹⁸ In light of the paucity of information and the lack of concordance

of study results, additional investigations are needed to determine if allergen specific IgE serum levels could serve as relevant biomarkers to predict the success of AIT in dogs.

We could only find one study reporting changes in allergen specific IDT results during AIT in dogs.¹⁷ In ten dogs with AD treated with SLIT, the IDT scores were found to vary erratically during treatment, and reaction scores were not significantly different after SLIT compared to those before.¹⁷ In our study, however, the GWS after rDf2 IDT were lower than before SCIT in two-thirds of dogs. As successful AIT appears associated, with time, with a reduction in allergen specific IgE serum titres,² it is logical to expect that the cutaneous reactivity to culprit allergens would also decrease over time. Further studies should determine if the documentation of reduced skin reactivity to allergens during sequential IDT would be of value to forecast favourable AIT outcomes in atopic dogs.

In a previous report, dogs with AD received "classic" SCIT and there was a significant rise in serum levels of the immunoregulatory cytokine IL-10 during treatment.¹⁶ Whether or not increases in IL-10 levels were of higher magnitude in dogs whose signs were reduced with AIT than in "non-responder dogs", was not addressed in that study. In our trial, changes in serum levels of the relevant cytokines IL-2, -4, -10 and interferon-gamma were inconsistent and often below the detection limit; this prevented their use as biomarkers of favourable treatment response. We suspect that the monovalent AIT protocol

used might have been too restrictive to lead to immunological changes of a magnitude high enough to be detectable in the peripheral circulation.

Throughout this study adverse events of rDf2-P SCIT were not seen, even in the two dogs that had exhibited allergic signs during sensitization to this allergen. The lack of adverse effects with this high-dose SCIT protocol appears more encouraging than during classic AIT in which minor adverse effects are often seen.⁸ It is possible that the conjugation to pullulan, which can reduce IgE responses to allergens,^{19,20} might have prevented access to antigenic epitopes and, hence, helped decrease the risk of immediate allergic reactions to the high doses injected.

In conclusion, we report herein that high-dose pullulan-conjugated monovalent rDf2 SCIT appears to reduce rapidly allergen induced skin lesions. It is associated with reduced, but not significantly different, allergen specific hypersensitivity and it seems safe. These observations warrant the performance of larger randomized controlled trials with atopic dogs sensitized to Df2. Whether or not this formulation would be of value in mite allergic dogs without detectable Df2-specific IgE is worth testing.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table 1. Evolution of serum cytokine levels during treatment.

Résumé

Contexte – L'immunothérapie d'allergène est actuellement la seule intervention proposée spécifiquement pour prévenir les crises après provocation allergénique. Derf 2 (Df2) de faible poids moléculaire est un allergène majeur chez les chiens japonais sensibilisés aux acariens de poussière de maison *Dermatophagoides farinae*.

Objectifs – Une étude expérimentale pilote, en aveugle, contrôlée contre placebo a testé l'efficacité de l'immunothérapie sous cutanée (SCIT) avec de hautes doses de Df2 recombinant conjuguée à rDf2-P (mal-totriose pullulan).

Méthodes – Huit beagle maltais atopiques ont été sensibilisés à rDf2 puis randomisés à SCIT avec rDf2-P (six chiens) ou placebo (2). L'immunothérapie a consisté en six injections hebdomadaires de doses croissantes (0.1–10.0 µg) de rDf2-P suivies par quatre injections mensuelles de 10 µg de cet allergène. Les provocations épicutanées de rDf2, les sérologies d'IgE spécifiques de rDf2 et la réactivité intradermique ainsi que les mesures des niveaux sériques de cytokines ont été réalisées au cours de l'étude.

Résultats – Les injections sous-cutanées de placebo n'ont pas modifié la réactivité cutanée après provocation par rDf2, alors que celle des chiens traités avec SCIT rDf2-P a disparu chez cinq des six chiens (83%) et a été diminuée pour un des six (17%). Au cours de la maintenance de la SCIT, les scores de lésions cliniques étaient significativement plus faibles chez les chiens recevant le SCIT comparé à ceux traités avec placebo. Cette amélioration clinique était accompagnée par une diminution concurrente, bien que non significative, d'une diminution des sérologies d'IgE spécifiques de rDf2 et de la réactivité intradermique immédiate. Les taux sériques de cytokines n'étaient pas concluants. Aucun événement indésirable n'a été observé avec SCIT de rDf2-P.

Conclusions et importance clinique – Le nouvel SCIT mono-allergène semble sûr et efficace pour réduire les lésions cutanées après provocation allergénique; d'autres tests sont nécessaires chez les chiens atteints de dermatite atopique spontanée.

Resumen

Introducción – La inmunoterapia con alérgenos es actualmente la única intervención que se propone para evitar específicamente exacerbaciones clínicas después exposición a alérgenos. La proteína de bajo peso molecular Der f 2 (Df2) es un alérgeno importante en los perros japoneses sensibilizados a *Dermatophagoides farinae* ácaros del polvo doméstico.

Objetivos – un estudio piloto, ciego, controlado con placebo para probar la eficacia de la inmunoterapia subcutánea (SCIT) con altas dosis de Df2 recombinante conjugada con pululano de maltotriosa (rDf2-P).

Métodos – Ocho perros atópicos cruce de Beagle y Maltés se sensibilizaron a rDf2 y luego fueron asignados al azar a SCIT con rDf2-P (seis perros) o placebo (2). La inmunoterapia consistió en seis inyecciones semanales de dosis crecientes (0,1-10,0 mg) de rDf2-P seguido de cuatro inyecciones mensuales de 10 mg de este alérgeno. Durante el estudio se evaluaron la reexposición epicutánea a rDf2, serología para IgE específica de rDf2 y reactividad intradérmica, así como mediciones del nivel de citoquinas en suero.

Resultados – Las inyecciones subcutáneas de placebo no alteraron la reactividad cutánea después de la reexposición a rDf2, mientras que la de los perros tratados con rDf2-P SCIT desapareció en cinco de los seis perros (83%) y se redujo en uno de seis (17%). Durante el mantenimiento del SCIT, las valoraciones de las lesiones de la piel fueron significativamente menores en los perros que recibieron SCIT en comparación con los tratados con placebo. Esta mejoría clínica fue acompañada por una disminución concurrente, pero no significativa, en serología de IgE específica para rDf2 y reactividad intradérmica inmediata. Los niveles séricos de citoquinas fueron poco informativos. No hubo eventos adversos observados con rDf2-P SCIT.

Conclusiones e importancia clínica – la nueva SCIT mono-alérgeno parece ser segura y efectiva para la reducción de lesiones en la piel después de la reexposición a alérgenos; que merece más estudios en perros con dermatitis atópica espontánea.

Zusammenfassung

Hintergrund – Die Allergen Immuntherapie ist momentan die einzige Intervention, die speziell empfohlen wird, um klinische Schübe nach einer Allergenprovokation zu verhindern. Der f2 (Df2) mit niedrigem Molekulargewicht ist ein Hauptallergen bei japanischen Hunden, die auf *Dermatophagoides farinae* Hausstaubmilben sensibilisiert sind.

Ziele – Eine Pilotstudie, geblindet und Plazebo-kontrolliert, zum Austesten der Wirksamkeit der subkutanen Immuntherapie (SCIT) mit hohen Dosen an rekombinantem Df2 konjugiert mit Maltotriose Pullulan (rDf2-P).

Methoden – Acht Malteser Beagle Mischlinge mit atopischer Dermatitis wurden auf rDf2 sensibilisiert und dann zufällig auf SCIT mit rDf2-P (sechs Hunde) und Plazebo (2) aufgeteilt. Die Immuntherapie bestand aus sechs wöchentlichen Injektionen bei zunehmender Dosis (0,1-10,0 µg) von rDf2-P gefolgt von vier monatlichen Injektionen mit 10 µg dieses Allergens. Epicutane rDf2 Provokationen, rDf2-spezifische IgE Serologie und Intradermale Reaktivität, sowie Serumzytokinmessungen wurden während der ganzen Studie durchgeführt.

Ergebnisse – Die subkutanen Injektionen des Plazebo änderten die Hautreaktivität nach Provokation mit rDf2 nicht, während jene bei fünf von sechs Hunden (83%), die mit rDf2-P SCIT behandelt worden waren, verschwand und bei einem von sechs Hunden (17%) reduziert war. Während der Erhaltungstherapie mit SCIT waren die Beurteilungen der Veränderungen bei Hunden, die SCIT erhielten, signifikant niedriger als bei jenen, die mit Plazebo behandelt worden waren. Die klinische Verbesserung wurde von einer gleichmäßigen, jedoch nicht signifikanten Abnahme der rDf2-spezifischen IgE Serologie und einer

intradermalen Sofortreaktion begleitet. Die Serumwerte der Zytokine ermöglichten keine Schlüsse zu. Es wurden mit rDf2-P-SCIT keine Nebenwirkungen beobachtet.

Schlussfolgerungen und klinische Bedeutung – Die neue mono-Allergen SCIT scheint sicher und wirksam zur Reduzierung der Hautveränderungen nach Allergenprovokation zu sein; diese Ergebnisse bedürfen bei Hunden mit spontaner atopischer Dermatitis weiterer Untersuchungen.

要約

背景 — アレルゲン免疫療法は、アレルゲン感作後の臨床症状の増悪を特異的に予防する唯一の方法として提唱されている。低分子Der f 2(Df2)はDermatophagoides farinaeハウスダストマイトに感作された日本の犬の主要抗原である。

目的 — マルトトリアースプルラン共役組み換えDf2(rDf2-P)の高用量皮下投与免疫療法(SCIT)の効果を検証する盲検プラセボ対照比較予備実験。

方法 — 8頭のマルチーズビーグル雑種のアトピー犬をrDf2で感作し、rDf2-P(6頭)あるいはプラセボ(2頭)によるSCITに無作為に振り分けた。免疫療法は、rDf2-Pを用量を増やしながら(0.1–10.0 µg)1週間ごとに計6回注射後、10 µgを1ヶ月ごとに計4回注射した。試験期間中は、rDf2の接触皮膚感作、rDf2特異的IgE血清学検査および皮内反応性検査、および血清中のサイトカイン測定を実施した。

結果 — プラセボの皮下注射はrDf2感作後の皮膚反応に変化を与えなかったが、rDf2-P SCITで治療した犬6頭中5頭(83%)で皮膚反応が消失し、1頭(17%)では減少した。SCITで維持治療中、SCIT治療群はプラセボ群と比較して、有意に低い皮膚病変スコアを示した。この臨床症状の改善は、rDf2特異的IgE血清学検査結果および即時型皮内反応性検査結果と、有意ではないが相関していた。血清中のサイトカイン値については、決定的な結果が得られなかった。rDf2-P SCITによる有害事象はなかった。

結論および臨床的重要性 — 新規の単一アレルゲンSCITは、アレルゲン感作後の皮膚病変を減少させるのに、安全かつ効果的であった。自然発生性のアトピー性皮膚炎に対する更なる試験が望まれる。

摘要

背景 — 過敏原免疫療法通过過敏原激发,可预防临床过敏发作,是目前唯一的特异性干预治疗方法。低分子量Der f 2(Df2)是使日本犬致敏的粉尘螨主要过敏原。

目的 — 试用、盲法、安慰剂对照法实验,用高剂量的与麦芽三糖支链淀粉共轭的重组Df2(rDf2-P),测试皮下免疫治疗(SCIT)的功效。

方法 — 八只马耳他犬异位性犬对rDf2敏感,用rDf2-P(6只犬)或安慰剂(2只)随机进行SCIT。逐渐增加Df2-P的剂量(0.1–10.0µg)每周注射六次这种过敏原进行免疫治疗,随后每月四次注射10µg。在整个研究中,进行经表皮rDf2激发,rDf2-特异性IgE血清学和皮内试验反应,以及血清细胞因子水平测量。

结果 — 皮下注射安慰剂的犬,在rDf2激发后没有改变皮肤反应,而用rDf2-P SCIT治疗的六只犬中的五只皮肤反应消失(83%),在六只中的一只皮肤反应减少(17%)。在SCIT维持期间,与使用安慰剂治疗的那些犬相比,接受SCIT的犬皮肤病变评分显著降低。这种临床改善同时, rDf2特异性IgE血清学反应和即时皮内反应伴随着并不显著的降低。无法确定细胞因子血清水平。使用rDf2-P SCIT没有出现副反应。

结论和临床意义 — 新的单过敏原SCIT显示,过敏原激发后能安全有效地减少皮肤病变;应该针对自发的异位性皮炎患犬做进一步测试。

Resumo

Contexto – Imunoterapia alérgeno específica é, atualmente, a única intervenção com o objetivo de especificamente prevenir crises após a exposição aos alérgenos. O alérgenos de baixo peso molecular Der f2 (Df2) é o principal alérgeno em cães japoneses sensibilizados ao ácaro da poeira doméstica *Dermatophagoides farinae*.

Objetivos – Ensaio piloto, cego, placebo-controle, experimentalmente testando a eficácia de imunoterapia subcutânea (ITSC) com altas doses de Df2 recombinante conjugado com maltotriose pullulan (rDf2-P).

Métodos – Oito cães maltês-beagle foram sensibilizados com rDf2 e depois tratados aleatoriamente com rDf2-P (seis cães) ou placebo (dois cães). A imunoterapia consistiu de seis injeções semanais com doses crescentes (0.1–10.0 µg) de rDf2-P seguidas por quatro injeções mensais de 10 µg deste alérgenos. Desafios epicutâneos com rDf2, sorologia IgE rDf2-específica e reação intradérmica, assim como a mensuração do nível sérico de citocinas, foram realizados ao longo do estudo.

Resultados – Injeções subcutâneas de placebo não alterou a reatividade cutânea após desafio com rDf2, enquanto em cães tratados com ITSC rDf2-P, esta desapareceu em seis cães (83%) e foi reduzida em um (17%). Durante a manutenção de ITSC, os escores de lesões foram significativamente menores em cães recebendo ITSC comparado com aqueles tratados com placebo. Esta melhora clínica foi acompanhada com um declínio concomitante, ainda que não significativo, na sorologia IgE rDf2-específica e reatividade intradérmica imediata. Os níveis de citocinas foram inconclusivos. Não ocorreram eventos adversos com ITSC rDf2-P.

Conclusões e relevância clínica – O novo monoalérgeno para ITSC aparenta ser seguro e efetivo na redução das lesões cutâneas após desafio alérgico. Este tópico merece estudos posteriores com cães com dermatite atópica espontânea.

Supplementary Table 1

	Interleukin-2 (pg/ml)			interferon-gamma (pg/ml)			interleukin-4 (pg/ml)			interleukin-10 (pg/ml)		
	Before	1 m post M-4	% change	Before	1 m post M-4	% change	Before	1 m post M-4	% change	Before	1 m post M-4	% change
PLA1	1,097	546	-50%	LLD	LLD	-	LLD	LLD	-	22	32	46%
PLA2	83	26	-69%	73	73	0%	LLD	LLD	-	97	60	-38%
SCIT1	LLD	LLD	-	LLD	LLD	-	LLD	LLD	-	27	31	16%
SCIT2	61	158	161%	LLD	78	-	2,159	1,835	-15%	34	23	-32%
SCIT3	45	LLD	-	LLD	LLD	-	LLD	LLD	-	17	17	4%
SCIT4	LLD	LLD	-	340	127	-63%	LLD	LLD	-	15	31	107%
SCIT5	85	436	413%	LLD	LLD	-	30	LLD	-	6	3	-44%
SCIT6	93	53	-43%	165	73	-56%	649	227	-65%	LLD	LLD	-

abbreviations: LLD: lower limit of detection; PLA: placebo, SCIT: subcutaneous immunotherapy