

Use of immunostimulatory liposome-nucleic acid complexes in allergen-specific immunotherapy of dogs with refractory atopic dermatitis – a pilot study

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Abstract This pilot study evaluated the effects of immunostimulatory liposome-plasmid-DNA complexes combined with specific allergens for immunotherapy of refractory canine atopic dermatitis. Seven dogs with previously diagnosed atopic dermatitis and unsatisfactory response to at least 12 months of conventional allergen-specific immunotherapy underwent a series of six intradermal injections (weeks 0, 2, 4, 6, 10 and 14), with patient-specific allergen extracts contained in cationic liposome-DNA complexes. Degree of pruritus was assessed on a visual analogue scale. Lesion scores were determined using the Canine Atopic Dermatitis Extent and Severity Index (CADESI) and medication usage was recorded at weeks 0 and 14. Canine cytokine mRNA expression in peripheral blood mononuclear cells collected prior to treatment and at the completion of the study was determined for IFN- γ , IL-4, TNF and IL-10 genes using quantitative reverse transcription competitive polymerase chain reaction. Repeated intradermal injections of specific allergens incorporated into liposome-nucleic acid complexes were well tolerated in all seven dogs. There was a significant improvement in pruritus scores ($P = 0.0277$) and concurrent significant decrease in IL-4 production ($P = 0.0428$) at the completion of the trial compared to pretreatment values. Medication scores, CADESI and production of other cytokines did not change significantly with treatment. These early results suggest that antigen-specific immunotherapy using a novel liposome-nucleic acid complex vaccine may be beneficial for treatment of established atopic dermatitis in dogs using lower antigen doses. Further investigations in larger numbers of dogs with earlier stages of disease are warranted.

INTRODUCTION

Canine atopic dermatitis is a common skin disorder in small animal practice.¹ Its prevalence seems to have increased during the last decades,^{1,2} similar to the increase observed in human allergic diseases.³ It may be treated with a variety of different medications such as glucocorticoids, antihistamines, fatty acid supplementation or shampoo therapy.⁴ However, none of these medications have been shown to reverse the underlying hypersensitivities. The only current specific treatment is allergen-specific immunotherapy. This therapy has a success rate of 50–70%, higher than that seen with antihistamine therapy or fatty acid supplementation.^{5–7} Adverse effects are also much less common than with glucocorticoid therapy. However, antigen-specific immunotherapy requires repeated injection of high doses of antigen mixtures and often takes several months to have a therapeutic effect. Moreover, more than 20–40% of patients fail to respond to treatment.⁶ Thus, there is a clear need for

more effective strategies to reverse the immune dysregulation that is proposed to underly the pathogenesis of canine atopic dermatitis.

T cells are critically involved in the pathogenesis of human atopic dermatitis and many of these T cells show a T helper (Th) 2 cytokine profile with increased expression of interleukin (IL)-4, IL-5 and IL-13. Successful immunotherapy in human atopic patients is associated with down regulation of IL-4 production.^{8,9} The cytokine profile of T cells in dogs with atopic dermatitis has been similarly characterized by an increase in production of Th2 cytokines.¹⁰ Immunization of mice using plasmid DNA-based vaccines reportedly induced a highly Th1-biased immune response and prevented development of Th2-biased immunity in a mouse model of atopy.¹¹ Allergens mixed with CpG oligodeoxynucleotides have also proved significantly more effective than vaccination with allergens alone in the reversal of a Th2-biased profile in mice.¹² The safety of immunostimulatory oligodeoxynucleotides conjugated to ragweed allergen has been shown in humans.¹³ Ragweed-immunostimulatory oligodeoxynucleotide conjugate immunotherapy of patients with allergic rhinitis has been shown to be significantly more effective than placebo treatment.¹⁴

We and others have previously shown that combining CpG oligonucleotides or plasmid DNA with liposomes

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markedly augments the immune stimulatory properties of these molecules.^{15,16} There have also been recent reports that liposome-nucleic acid complexes can be combined with protein or peptide antigens and used effectively as a novel vaccine adjuvant.¹⁷ The purpose of this study was to evaluate a liposome-nucleic acid-based vaccine for allergen-specific immunotherapy of canine atopic dermatitis.

MATERIALS AND METHODS

Study design

Seven dogs with nonseasonal atopic dermatitis diagnosed by history, clinical signs and appropriate exclusion of differential diagnoses^{18,19} such as food adverse reaction or scabies were included in the study. All protocols for this study were approved by the Institutional Animal Care and Use Committee at Colorado State University. All dogs enrolled in the study had multiple positive reactions on intradermal testing, and had previously been on continuous allergen-specific immunotherapy using a combination of selected allergen extracts based on intradermal testing (Greer Veterinary Allergy Products, Greer Laboratories, Lenoir, NC, USA) for at least 12 months with incomplete or no clinical response. All still showed residual pruritus and most were on additional antipruritic therapy. Dogs were evaluated clinically before and 14 weeks after entering the study. Pruritus was marked by the owner on a visual analogue scale from 0 to 30. Lesions were determined by a clinician using the Canine Atopic Dermatitis Extent and Severity Index (CADESI) and medication scores were recorded as previously described.^{20,21} Briefly, no concurrent medication scored 0 points, shampoo therapy scored 5 points and antihistamines and/or fatty acid supplementation scored 10 points. The score for glucocorticoids was determined by the dose used. A calculated daily average dose of 1 mg kg⁻¹ or more of prednisone or prednisolone scored 40 points, between 0.5 mg kg⁻¹ and 1 mg kg⁻¹ daily 30 points, between 0.2 mg kg⁻¹ and 0.5 mg kg⁻¹ daily 20 points and less than 0.2 mg kg⁻¹ 10 points.

Preparation of allergen-specific vaccines

Each dog received its own, individually tailored, allergen extract mixture both before and during the study. The allergen extracts contained between 20 000 and 40 000 PNU mL⁻¹ (this was equivalent to 50 µg of protein measured using a commercially available assay (BCA assay, Pierce Biotechnologies, Rockford, IL, USA) that determines the protein concentration of the test substance by comparison to a standard curve generated using bovine serum albumin). The allergens were added to preformed liposome and plasmid DNA complexes, which were prepared as described previously.¹⁶ Briefly, sterile solutions of cationic liposomes (a 20 mM solution of DOTIM {octadecenoyloxy{ethyl-2-heptadecenyl-3-hydroxyethyl} imidazolinium chloride

and cholesterol), in a 1 : 1 molar ratio were prepared and the liposomes were extruded through a final filter diameter of 200 nm. Liposome-DNA complexes were formed just prior to injection by gently mixing cationic liposomes with plasmid DNA at a ratio of 16 nmol lipid per 1 µg DNA in 5% dextrose in water at room temperature. The final plasmid DNA concentration in the complexes was 100 µg DNA per mL. Plasmid DNA was prepared by the modified alkaline lysis procedure, followed by polyethylene glycol precipitation, as described previously.¹⁶ The plasmid DNA used in these studies did not contain a gene insert and served only as a source of CpG sequences for vaccine adjuvant purposes. The particular plasmid vector used in these studies contained 21 individual CpG motifs.¹⁶

Immediately prior to vaccination, 0.3 mL of liposome-DNA complexes were prepared and mixed with allergen extract by gentle pipetting. The amount of allergen used represented 10% of the original dose of allergen the dogs had previously been receiving as maintenance dose for conventional allergen-specific immunotherapy. The resulting vaccine was divided into two equal portions and then injected intradermally within 15 min after preparation. The injections were given intradermally in two sites over the dorso-lateral thorax, using a 25-gauge needle. Injections were administered at 0, 2, 4, 6, 10 and 14 weeks.

PBMC separation and stimulation

Six to 10 mL of heparinized whole blood was collected from each dog before and 14 weeks after initiation of therapy. Peripheral blood mononuclear cells (PBMC) were separated within 2 h of sample collection using a commercially available lymphocyte separation medium (LSM Lymphocyte Separation Medium, ICN Biomedicals, Aurora, OH, USA) according to the manufacturer's directions. The PBMC were then washed, aliquotted, and stored in cell freezing medium in liquid nitrogen until batch analysis. Two mL of PBMC were thawed and resuspended in Modified Eagle's Medium (MEM) (Minimal Essential Medium, Gibco-Invitrogen, Grand Island, NY, USA) containing 10% fetal bovine serum, 0.0375% bicarbonate, antibiotics (100 µg mL⁻¹ penicillin and 100 µg mL⁻¹ streptomycin), L-glutamine and essential and nonessential amino acids.

In an aliquot with a 1 : 1 dilution of Trypan blue, cell counts were adjusted for 106 live cells mL⁻¹. Cells were all greater than 90% viable. PBMC were then incubated at a final concentration of 1 × 10⁶ cells mL⁻¹ at 37 °C with 5% CO₂. PBMC were incubated in 12-well plates, with 2 mLs of cells per well. PBMC were subjected to the following treatments: incubation with media only for 4 h; incubation with phorbol myristate acetate (PMA: 10 ng mL⁻¹) plus ionomycin (500 ng mL⁻¹) (PMA I⁻¹) for the standard 4 h; incubation in media only for 24 h; and incubation in medium plus 50 µg mL⁻¹ of the specific allergen extract (determined by skin testing and identical to the individual extract used for allergen-specific immunotherapy) for each

patient for 24 h. The latter incubation time was based on preliminary studies. Cells were harvested by scraping the bottom of the well with a sterile pipette tip, aspirating contents of the well, and washing three times with sterile PBS before RNA extraction.

RNA isolation and cDNA synthesis

Total RNA was extracted from cultured PBMC immediately after harvest using a commercially available kit (RNeasy Mini Kit, Qiagen, Inc., Valencia, CA, USA) according to the manufacturer's directions. Any residual genomic DNA was removed with DNase I treatment (RNase-Free DNase Set, Qiagen, Inc.). The final product was eluted into 30 µL of DEPC-treated water. cDNA was synthesized from the total RNA using 10 µL of RNA, random primers (Random primers, Invitrogen, Carlsbad, CA, USA), dNTP mix (2.5 mM each dNTP), MMLV reverse transcriptase (MMLV Reverse Transcriptase, Invitrogen), and an RNase inhibitor (RNaseOUT Recombinant Ribonuclease Inhibitor, Invitrogen). The final product was brought up to a volume of 60 µL in DEPC-treated water and stored at -80 °C.

Quantitative PCR with real-time TaqMan(R) primers

TaqMan primer probe mixes were purchased from Dr Christian Leutenegger (Lucy Whittier Molecular Core Facility – UC Davis – TaqMan® Service, Davis, CA, USA) including primers and probes for canine GAPDH, interleukin (IL)-4, IL-10, interferon-gamma (IFN-γ), and tumour necrosis factor-alpha (TNF). The probes were labelled at the 5' and 3' ends with a reporter fluorescent dye, FAM, and a quencher dye, TAMRA, respectively. Amplifications were carried out in duplicate in 25-µL reaction mixtures containing (final concentration) 12.5 µL Mastermix (TaqMan® Universal PCR Master Mix, Applied Biosystems, Foster City, CA, USA), 0.5 µL (400 nM) of each primer, 0.2 µL (80 nM) of probe, 6.3 µL PCR grade water, and 5 µL template cDNA. Thermocycler conditions were as follows: 2 min at 50 °C, 10 min at 95 °C, and 40 cycles of 15 s at 95 °C followed by 1 min at 60 °C. Results were analysed using the instrument software (version 1.0). The positive threshold (threshold cycle [C_T]) was set at 10 times the standard deviation of the baseline fluorescence. Relative quantification was carried out using the comparative C_T method.²² Samples were normalized for amount of starting template in each reaction using canine GAPDH. Expression of the gene of interest was compared to the pretreatment, unstimulated cell population for each dog (calibrator sample).

Statistical evaluation

Values for CADESI, pruritus and medication scores at the beginning and end of the study were compared by means of a paired *t*-test. The increase or decrease of cytokine expression levels in PBMC samples obtained at the end of the study was calculated and compared to

the baseline values obtained at the beginning of the study, using a paired *t*-test.

RESULTS

All seven dogs enrolled completed the full 14-week study. Injections were well tolerated and with one exception no adverse effects were noted. One dog was inadvertently treated with five times the intended dose of allergen in the liposome-nucleic acid complex vaccine during the first injection. This dog developed a rapid increase in pruritus that improved again after 3–4 days. The dog had received a maintenance dose of 0.2 mL allergen extract prior to the study as initial administrations of 1.0 mL had caused dramatic increases in pruritus for several days after each injection. Subsequent injections were administered with the correct allergen dose and further adverse effects were not seen. With the exception of this dog, owners did not report adverse effects, though in some dogs, small, nonpruritic, nonpainful, intradermal papules could be palpated at the previous injection site.

The primary end points for this study were effects on degree of pruritus and lesion scores. At the completion of the study there was a significant improvement in mean pruritus scores (Fig. 1), with values decreasing from 15.7 ± 7.5 pre treatment to 9.4 ± 8.3 post treatment ($P = 0.0277$). Although the mean CADESI scores (Fig. 2) decreased numerically (from 30 ± 25 pre treatment to 23.2 ± 23.2 post treatment), the decrease was not significant. Similarly, the medication scores (Fig. 3) also decreased numerically, from 12.9 ± 7 to 10.7 ± 11.3 , but these changes were also not statistically significant. Improvement by more than 50% in pruritus scores was noted in 3/7 dogs, while the CADESI scores improved by more than 50% in 3/7 dogs (one of which also had 50% improvement in pruritus scores).

To assess the effects of the allergen-specific vaccine on cytokine profiles of PBMC from treated dogs, we measured cytokine mRNA levels pre and post treatment. Mean values of the pre- and poststimulation mRNA levels are given in Table 1. In one dog, the pretreatment unstimulated control did not show any expression of IL-4 mRNA; in this case the stimulated sample was used as the calibrator sample. When PBMC were re-stimulated with their specific antigens *in vitro* for 24 h, we observed a significant ($P = 0.0428$) decrease in IL-4 mRNA levels (from 3.8 ± 4.5 in pre treatment PBMC to 0.3 ± 0.7 in post treatment PBMC) (Fig. 4). In contrast, values for IFN-γ, TNF, or IL-10 production in PBMC re-stimulated with allergen did not change significantly when pre- and post-treatment samples were compared. Stimulation of PBMC with the agonists PMA and ionomycin did not reveal statistically significant differences in any of the four cytokines evaluated. These results suggest that immunotherapy with the appropriate allergens reformulated in a novel adjuvant was capable of reversing at least

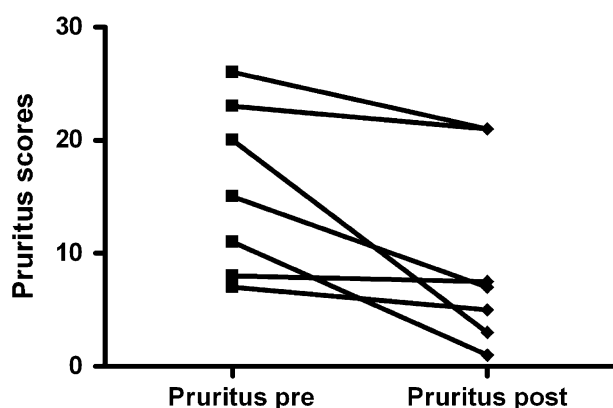


Figure 1. Pruritus scores before and after 14 weeks of treatment with allergen-specific immunotherapy and immunostimulatory DNA sequences.

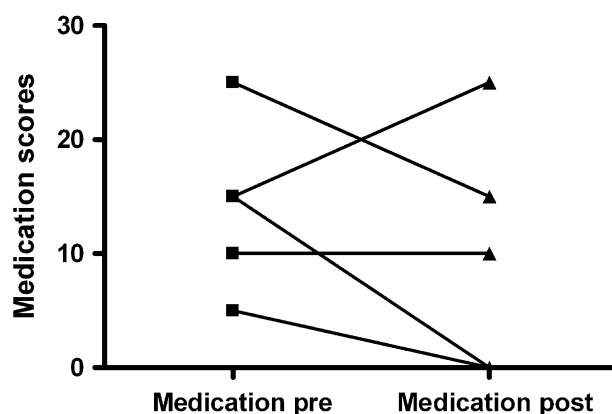


Figure 3. Medication scores before and after 14 weeks of treatment with allergen-specific immunotherapy and immunostimulatory DNA sequences.

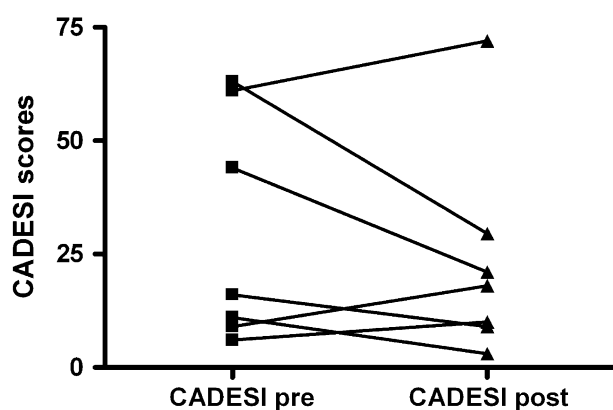


Figure 2. CADESI scores before and after 14 weeks of treatment with allergen-specific immunotherapy and immunostimulatory DNA sequences.

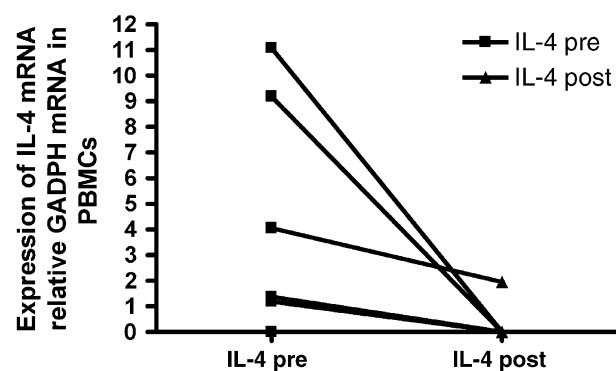


Figure 4. IL-4 production before and after 14 weeks of treatment with allergen-specific immunotherapy and immunostimulatory DNA sequences.

partially the Th2 phenotype of the allergen-specific T cells in the atopic dogs in this study.

DISCUSSION

We report here the results of a pilot study using a novel therapeutic combining allergen-specific immunotherapy and liposome-plasmid DNA complexes for therapy of refractory atopic dermatitis in dogs. There is increasing interest in the application of new vaccine approaches to the management of chronic atopic disease, given the increasing importance of these diseases in both humans and dogs. Allergic diseases in humans have

increased dramatically over the last decades, from 3% in 1946 to 12% in 1972. Currently, up to 30% of individuals in some populations are affected.³ Similarly, an increase in patients with canine atopic dermatitis from 7% in 1981² to 12% in 1990¹ has been observed in North-east America. A variety of drugs such as glucocorticoids,²³ cyclosporin,²⁰ antihistamines,²⁴ fatty acids^{21,25,26} or combinations thereof^{27–29} have been used for the treatment of atopic dermatitis: a comprehensive review has recently been published.⁴ These treatments are symptomatic and evidence of long-lasting remission after cessation of therapy has been scarce.³⁰

Allergen-specific immunotherapy can lead to desensitization in patients with atopic asthma,^{31,32} rhinitis¹⁴

Table 1. Mean cytokine mRNA levels of PBMC before and after treatment with allergen-specific immunotherapy and immunostimulatory DNA sequences

	Control		PMA I ⁻¹		Allergen	
	Pre	Post	Pre	Post	Pre	Post
IL-4	0.71 ± 0.49	0	1.8 ± 1.01	0.59 ± 0.46	3.84 ± 4.54	0.28 ± 0.74
IL-10	1	1.43 ± 2.15	1.12 ± 0.67	11.19 ± 25.85	0.55 ± 1.16	0.12 ± 0.14
IFN-γ	1	3 ± 4.51	1.28 ± 0.6	1.02 ± 0.71	2.15 ± 3.79	0.98 ± 0.85
TNF	1	2.13 ± 2.49	2.15 ± 1.22	1.73 ± 1.45	0.9 ± 0.54	0.65 ± 1.14

and atopic dermatitis in humans³³ and dogs.⁶ However, allergen-specific immunotherapy requires repeated subcutaneous injections and may take months before clinical improvement can be observed.⁶ The success rate of conventional immunotherapy in dogs varies from 50 to 70%.⁵⁻⁷ Thus, there are a number of patients that may not benefit from this type of therapy. The reasons for treatment failure are not known, but in some cases at least it may be related to the means by which the allergens are presented to the immune system. Therefore, improvements in delivery and formulation of the allergens may be expected to improve treatment outcomes, particularly in light of the recent success of incorporating CpG adjuvants for allergen immunotherapy in mouse models^{34,35} and clinical trials in human medicine.^{13,14}

Allergic diseases have been characterized as being driven by Th2-biased T-cell responses, accompanied by increased production of IL-4, IL-5 and IL-13 in mice and humans.³⁶⁻³⁸ In chronic lesions, increased concentrations of IFN- γ have also been reported. Evidence for IL-4 production in the skin of atopic dogs was first reported by Olivry.³⁹ A more recent study showed results very similar to the findings in humans and mice, where IL-4 and TNF mRNA levels were increased in atopic dog skin. In addition, in the lesional skin of these dogs IFN- γ was also elevated.¹⁰

Recent studies suggest that incorporation of immunostimulatory sequences (e.g. CpG oligodeoxynucleotide) together with allergen-specific immunotherapy may improve the ability of allergen immunotherapy to suppress or redirect Th2-biased hypersensitivities. For example, immunostimulatory CpG containing oligodeoxynucleotides were shown to inhibit the development of allergic conjunctivitis and asthma in mouse models.^{34,35} Conjugation of CpG oligodeoxynucleotides to allergen proteins were shown to result in reduced allergenicity and increased immunogenicity in another study.⁴⁰ The results of the first human clinical trials utilizing CpG oligodeoxynucleotides together with allergens have also been encouraging.^{13,14}

The purpose of the present study was to determine whether a similar approach using a potent Th1-biasing immune modulator might be effective clinically in dogs with long-standing atopic dermatitis. Unlike previous studies, however, we utilized complexes of liposomes and noncoding plasmid DNA to augment Th1 responses, in lieu of CpG oligonucleotides. We have previously shown that liposome-DNA complexes are extremely potent activators of innate immunity and release of Th1 cytokines such as IFN- γ .¹⁶ Others have shown that complexes of liposomes with CpG oligonucleotides are also much more potent than CpG oligonucleotides alone.¹⁵ Moreover, liposome-DNA complexes are also extremely effective vaccine adjuvants and are capable of significantly inhibiting established allergic lung disease in mice (S. W. Dow; unpublished data).

In this pilot study, a mixture of protein allergen extracts and liposome-DNA complexes was used for

allergen-specific immunotherapy. Although this mixture had to be prepared for each dog immediately prior to injection, the procedure was simple, rapid and allowed the use of individualized doses of different allergen extracts. Six injections were administered, similar to a protocol used in humans.¹⁴ The dose of allergen extract used to immunize dogs in this study was decreased by 90% from the dose used in conventional immunotherapy, based on the known prior potency of the liposome-DNA complex vaccines. In one dog, where the dose initially was not decreased appropriately, injection led to rapid increase of pruritus with a decrease after 3–4 days. The degree of pruritus was similar to that which the owner had noted previously after injection of 1 mL of allergen extract. Subsequent injections were given at the appropriately adjusted dose with no more adverse effects. Therefore, it is likely that dose reduction is important when combining the allergen extract with liposome-DNA complexes.

This study consisted of a small number of dogs with atopic dermatitis on allergen-specific immunotherapy for at least 12 months with no or partial improvement. Because the dogs were not well controlled clinically, cessation of all medication for a period before inclusion in and during the study was considered unethical and would not have been accepted by the owners. Instead, the enrolled patients in our study were allowed to remain on their current medications and the use of medications was recorded at the start and the end of the study using a previously used scoring system.²¹ Pruritus scores improved significantly in this small group of dogs during the 14 weeks of treatment. Although CADESI and medication scores decreased as well, the change was not statistically significant. In a recent, double-blinded, placebo-controlled human study using 6-weekly injections of allergen extract linked to oligodeoxynucleotides, symptom scores continued to decrease for 12 months after the last administration.¹⁴ In another human study, a short series of injections with Amb a 1 immunostimulatory oligodeoxynucleotide conjugate led to improvement lasting for two allergy seasons (P.S. Creticos, personal communication, 2003). Whether dogs included in this study will continue to improve in a similar fashion is currently unknown. In previous studies a 5 per cent reduction from baseline of pruritus or lesional scores was considered to represent a clinically relevant threshold above which both clinicians and owners would be satisfied with treatment effect.²⁰ Thus, in addition to the mean decrease of pruritus, CADESI and medication scores, we determined the number of dogs improving by more than 50% in pruritus and/or lesions scores. Three of the seven dogs improved by more than 50% in their lesional scores and 3/7 in their pruritus scores. These dogs were classified as not responding to immunotherapy prior to administration of the liposome-DNA complexes. Thus, the noted improvement in mean pruritus, lesion or medication scores in combination with 40% of the dogs' individual scores improving by > 50% assumes greater significance than if newly diagnosed atopic patients were being evaluated.

Allergen-specific immunotherapy in combination with CpG-containing oligodeoxynucleotides has previously led to a clinical response with a concurrent decrease in concentrations of Th2 cytokines such as IL-4 and IL-5 in mice¹² and humans.¹⁴ To the authors' knowledge, similar studies documenting a change in cytokine profile following allergen-specific immunotherapy in dogs have not been reported. CpG oligodeoxynucleotides bind to the Toll-like receptor (TLR) 9 within the endosomal compartment of dendritic cells, macrophages, and B cells. Binding to the TLR9 elicits functional maturation of antigen-presenting cells (particularly dendritic cells) and release of cytokines such as IFN- γ , TNF, IL-6 and IL-12, as well as up-regulation of the expression of costimulatory molecules such as CD40, CD80, CD86, B7.1 and B7.2.^{41,42} Th2 responses are at the same time reduced when cells are activated by CpG oligonucleotides; for example, eosinophil production in the bone marrow is inhibited, and IL5 production is reduced.⁴³ A decrease in IL-4 receptor expression and in IL-4-dependent IgE synthesis in an IL-12- and IFN-dependent manner has also been observed.⁴⁴ Altogether, these findings suggest that it may be possible to switch the Th2 phenotype by administration of immunostimulatory nucleic acids such as CpG containing oligodeoxynucleotides or plasmid DNA in humans and rodents.

In the present study, we observed that after incubation with allergen, cytokine production by PBMC was altered. A significant decrease of IL-4 production from baseline after 14 weeks of therapy suggests that exposure to allergen in the context of a potent Th1-biasing immune stimulant such as liposome-DNA complexes may in fact be capable of altering the Th2 phenotype in dogs. In general, expression of the other cytokines by PBMC decreased as well, although TNF increased in one patient and IFN and IL-10 increased in two patients each. With the exception of the decrease in IL-4 production, there was no consistent pattern of changes in cytokine expression between patients but interpretation is difficult due to the small number of patients in the trial. Further studies with a larger number of patients are needed to evaluate the influence of immunotherapy on cytokine production and possible correlations between clinical improvement and cytokine production.

It is important to note that all seven dogs enrolled in this study had failed conventional immunotherapy that had been administered for a period of at least 1 year. Moreover, the dogs were re-immunized with the same original allergens, so the only change was the addition of the liposome-DNA adjuvant. Forty per cent of the treated dogs had at least a 50% improvement in overall clinical scores. Although these preliminary results appear exciting, readers should be aware that this was an uncontrolled study and therefore could have been subject to placebo effects in relation to the improvements in pruritus. Hence, large scale, controlled studies are clearly needed before the precise efficacy of this mode of treatment can be established.

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Résumé Cette étude pilote a évalué les effets de complexes liposome-plasmides-DNA immunostimulants, couplés avec des allergènes spécifiques, pour l'immunothérapie de cas réfractaires de dermatite atopique canine. Sept chiens présentant une dermatite atopique et ne répondant pas à une immunothérapie conventionnelle après 12 mois de traitement ont reçu une série de 6 injections intradermiques (semaines 0, 2, 4, 6, 10 et 14) des allergènes spécifiques couplés à des complexes cationiques liposome-DNA. Le degré de prurit a été évalué par une échelle linéaire visuelle. Les scores lésionnels ont été notés avec le CADESI et les prises médicamenteuses ont été notées les semaines 0 et 14. L'expression d'ARNm des cytokines dans les cellules mononucléées du sang périphérique a été réalisée avant traitement et après l'essai (dosage d'IFN- γ , IL-4, TNF et IL-10 en utilisant une reverse transcription compétitive polymérase chain reaction). Les injections intradermiques répétées des allergènes spécifiques incorporés dans les complexes de liposome-acide nucléique ont été bien tolérées chez les 7 chiens. Une amélioration statistiquement significative du prurit ($P=0.0277$) et une diminution parallèle de la production d'IL-4 ($P=0.0428$) à la fin du traitement ont été notés par rapport aux valeurs pré-traitement. Les scores de consommation médicamenteuse, le CADESI et les autres cytokines n'ont pas été modifiés significativement après le traitement. Ces résultats préliminaires suggèrent que l'immunothérapie spécifique d'antigènes, utilisant un nouveau vaccin à base d'un complexe de liposomes et d'acide nucléique, peut être intéressante pour le traitement de la dermatite atopique canine. Des études supplémentaires sur un grand nombre d'animaux, souffrant d'une maladie moins évoluée, sont nécessaires.

Resumen Este estudio piloto evaluó los efectos inmunoestimulantes de los complejos liposoma-plásmido-DNA combinados con alérgenos específicos para la inmunoterapia de la dermatitis atópica canina refractaria. Siete perros con una dermatitis atópica previamente diagnosticada y con respuesta no-satisfactoria a un mínimo de 12 meses de tratamiento con una inmunoterapia alérgeno-específica convencional fueron sometidos a series de 6 inoculaciones intradérmicas (semana 0, 2, 4, 6, 10 y 14) con extractos de alérgenos paciente-específicos contenidos en complejos catiónicos liposoma-DNA. El grado de prurito fue evaluado con una escala análoga visual. Las puntuaciones de la lesión fueron determinadas utilizando el Índice de Gravedad y Extensión de la Dermatitis Atópica Canina (CADESI) y la medicación usada fue registrada en las semanas 0 y 14. La expresión de mRNA de citoquina canina en células mononucleares de sangre circulante recogidas antes del tratamiento y al final del estudio fue determinada para genes de IFN- γ , IL-4, TNF y IL-10 utilizando una PCR competitiva de la transcriptasa inversa cuantitativa. Las inoculaciones intradérmicas repetidas de alérgenos específicos incorporados en complejos de ácidos nucleicos-liposomas fueron toleradas en 7 perros. Hubo una mejora significativa de los registros de prurito ($P=0.0277$) y una disminución concomitante significativa en la producción de IL-4 ($P=0.0428$) al final de la prueba comparado con los valores anteriores al tratamiento. Los registros de la medicación, del CADESI y la producción de otras citoquinas no cambiaron significativamente con el tratamiento. Estos resultados iniciales sugieren que una inmunoterapia antígeno-específica utilizando una novedosa vacuna consistente en un complejo de liposoma-ácido nucleico puede ser beneficiosa para el tratamiento de una dermatitis atópica establecida en perros que utilicen dosis más bajas de antígeno. Son necesarias más investigaciones con un mayor número de perros en estadios anteriores de la enfermedad.

Zusammenfassung Diese Pilotstudie überprüft die Wirkungen von mit spezifischen Allergenen kombinierten immunstimulatorischen Liposom-Plasmid-DNA-Komplexen auf die Immuntherapie bei refraktärer caniner atopischer Dermatitis. Sieben Hunde mit zuvor diagnostizierter atopischer Dermatitis und unbefriedigendem Behandlungserfolg nach mindestens zwölfmonatiger konventioneller, allergenspezifischer Immuntherapie wurden einer Serie von sechs intradermalen Injektionen (Woche 0, 2, 4, 6, 10 und 14) mit patienten-spezifischen Allergenextrakten in kationischen Liposom-DNA-Komplexen unterzogen. Der Grad des Juckreizes wurde mit einer visuellen Analogskala beurteilt. Punktzahlen für Läsionen wurden mit CADESI (Canine Atopic Dermatitis Extent and Severity Index) bestimmt und eingesetzte Medikamente wurden an Woche 0 und 14 aufgezeichnet. Die Expression von Cytokin-mRNA in peripheren mononukleären Blutzellen, die vor Beginn der Behandlung und nach Beendigung der Studie gesammelt wurden, wurde für IFN- γ , IL-4-, TNF- und IL-10-Gene durch quantitative, kompetitive Reverse-Transkriptions-PCR bestimmt. Wiederholte intradermale Injektionen von in Liposom-Nukleinsäuren-Komplexen inkorporierten spezifischen Allergenen wurde von allen 7 Hunden gut toleriert. Es gab eine signifikante Verbesserung der Juckreiz-Punktzahlen ($P=0.0277$) und eine gleichzeitige signifikante Verringerung der IL-4-Produktion ($P=0.0428$) bei Beendigung des Versuches im Vergleich zu den Werten vor Beginn. Die Punktzahlen für Medikamente, CADESI und die Produktion anderer Cytokine veränderten sich mit der Behandlung nicht signifikant. Diese ersten Resultate lassen vermuten, dass antigen-spezifische Immuntherapie mit einer neuartigen Liposom-Nukleinsäuren-Komplex-Vaccine zur Behandlung etablierter atopischer Dermatitis bei Hunden und Verwendung niedrigerer Antigendosen von Nutzen sein können. Weitere Forschungen bei einer größeren Anzahl von Hunden mit früheren Krankheitsstadien sind notwendig.