

Changes in cytokine profiles following treatment with food allergen-specific sublingual immunotherapy in dogs with adverse food reactions

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Background – Food allergen-specific sublingual immunotherapy (FA-SLIT) is considered to be a novel, safe and effective approach in dogs with adverse food reactions (AFR).

Hypothesis/Objectives – To investigate changes in key cytokines associated with FA-SLIT.

Animals – Eleven dogs with confirmed AFR.

Methods – Participants received either dose escalation of FA-SLIT or placebo over a six month period. Oral food challenge was performed at the beginning and end of the study, along with clinical examinations and collection of skin surface bacterial cytology and blood. Peripheral blood mononuclear cells were stimulated with the culprit food antigen. ELISA methods were used to quantify Interleukin (IL)-10, IFN- γ , IL-4 and IL-17A in the supernatant of stimulated cells.

Results – IL-10 and IFN- γ levels were significantly increased at the end of the study in the treatment group (T), compared with the placebo group (P), whereas no changes were found in IL-4 levels. IL-17A levels were decreased in both groups (but more profoundly in T). Bacterial scores on the skin were positively correlated with IL-17A and inversely correlated with IL-10 concentrations. Interleukins were not correlated with clinical scores.

Conclusion and clinical importance – FA-SLIT may modulate the allergic response toward Th1 and Treg cell phenotypes, and induction of tolerance in dogs with AFR. Therefore, FA-SLIT may be a tool to desensitize dogs with AFR. However, more data on a larger number of cases and a broader panel of cytokines are needed to corroborate these findings, and to elucidate the mechanism of action for responses to FA-SLIT by dogs with AFR.

Introduction

Currently, the only known treatment for adverse food reactions (AFR) is avoidance of offending allergens and administration of anti-inflammatory medications on accidental exposure. Given the prevalence of AFR and the significant impairment in the quality of life for both dogs and owners, the development of a safe and efficacious therapy targeting AFR is highly desirable.

Sublingual immunotherapy (SLIT) is a novel approach used in the treatment of people with food allergy by inducing desensitization and eventually tolerance.¹ With SLIT, small amounts of allergen extract are delivered sublingually as drops or tablets. Although the exact mechanisms of desensitization with allergen-specific immunotherapy are still not fully understood, it is known that skewing of T-cell responses from allergen-specific effector T cells toward regulatory T cells (Tregs) is an essential event in the

development of healthy immune responses to allergens and is correlated with successful allergen-specific immunotherapy (ASIT) in people.² Interleukin (IL)-10 is thought to be a key cytokine driving the differentiation of Tregs, suppress Th2 cells, mast cells, basophils and eosinophils, and inducing a class switch from IgE to IgG and IgA production by B cells.² In humans, other cytokines also have been evaluated as candidate biomarkers to monitor immunotherapy, but the results are inconsistent. IL-5 and IL-13 decrease, TGF- β transiently increases, IFN- γ increases but not consistently, IL-2 does not change, and IL-4 and IL-17 are undetectable.^{1,3} There are numerous studies available on immunotherapy for food allergy in humans, but studies in veterinary medicine are lacking. We have reported on the results of a study of food allergen (FA)-SLIT as a treatment for AFR in dogs.⁴ It was observed that FA-SLIT had a favourable safety profile associated with decreased pruritus and clinical signs in some subjects. In the present study we aimed to investigate if the clinical improvements were accompanied by changes in the allergic cytokine response. In order to understand the role of IL-17A in dogs with AFR, we assessed the correlation between IL-17A and clinical lesions, pruritus and bacterial overgrowth.

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Materials and methods

Animals and sampling

Dogs were enrolled as reported previously.⁴ Briefly, dogs with proven AFR and no other concurrent allergic conditions were randomized to receive either treatment (T) with FA-SLIT ($n = 7$) or placebo (P) consisting of glycerinated saline ($n = 6$). During the study dogs were fed a restrictive diet. Clinical signs were provoked by feeding the culprit diet before and at the end of the study. Severity of clinical signs were assessed with a pruritus Visual Analog Scale (PVAS) and the fourth iteration of the Canine Atopic Dermatitis Extent and Severity Index (CADESI-04).^{5,6} The treatment was continued daily for at least six months with fortnightly dose escalations. Peripheral blood samples were collected by cephalic or jugular venipuncture during the two provocative food exposure phases (before and after

treatment). Concurrently, smears were obtained by direct impression of skin from the axillary and groin areas of the dogs. Samples were stained with a Romanowsky-type stain (Hemacolor, Merck; Darmstadt, Germany) and evaluated using high power microscopy fields (HPF: $\times 1,000$ magnification) for the presence of bacteria. Presence of bacteria and number of bacteria engulfed by neutrophils was evaluated empirically using a 0–4 severity scale (0, none seen; 1, ≤ 1 /HPF; 2, 1–5/HPF; 3, 5–10/HPF; 4, ≥ 10 /HPF).

Lymphocyte isolation, stimulation and cytokine ELISA

Isolation of peripheral blood mononuclear cells (PBMCs) from whole blood was performed as described previously.⁷ Cells were then cultured in triplicate in flat-bottomed 96-well microtitre plates at 5.0×10^8 cells/mL with allergen extracts (pork, chicken, beef, cow's

Table 1. Cytokine [interleukin (IL)-10, IFN- γ and IL-17A, IL-4] concentration in the supernatant of allergen-stimulated peripheral blood mononuclear cells of the placebo and treatment group before (Pre) and after (Post) the study.

	<i>n</i>	IL-10 [†]		IFN- γ [†]		IL-4 [†]		IL-17A [†]	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post
Placebo	6	0	0	810 $\pm$ 839	786 $\pm$ 897.2	43 $\pm$ 65	19.6 $\pm$ 55.5	22 $\pm$ 18	7.1 $\pm$ 20*
Treatment	5	0	33 $\pm$ 51*	103 $\pm$ 198.2	321 $\pm$ 387*	39.6 $\pm$ 89.7	43.4 $\pm$ 99.1	134.4 $\pm$ 100.4	17.3 $\pm$ 57.2*

*Statistically significant.

[†]Mean $\pm$ SD cytokine concentration (pg/mL).

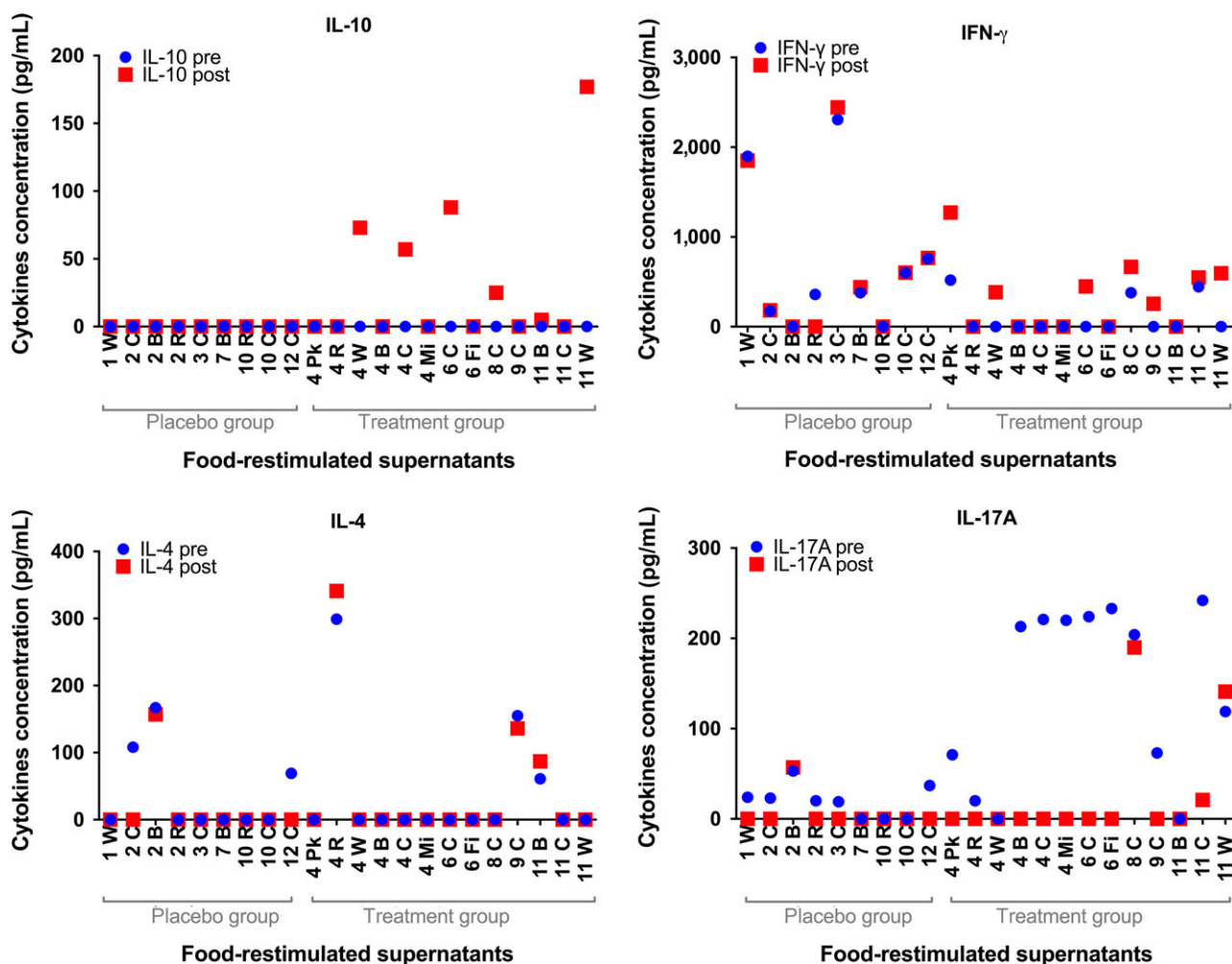


Figure 1. Cytokine concentrations in the supernatant of food allergen-stimulated peripheral blood mononuclear cells of individual dogs at the beginning (pre) and end (post) of treatment. Values for Case 5 are missing because the dog was withdrawn during the study. B, beef; C, chicken; Fi, fish; Mi, cows milk; Pk, pork; R, rice; W, wheat.

milk, fish, rice, wheat and corn at concentrations of 25 µg/mL; Greer laboratories; Lenoir, NC, USA), concanavalin A (10 µg/mL; Amersham Pharmacia Biotech; Freiburg, Germany) or plain media. The PBMCs were then incubated at 37°C in a 5% CO₂ humidified atmosphere for five days. The supernatant of the food allergen-stimulated cells was collected and the concentration of IL-10 (Treg), IFN-γ (Th1), IL-4 (Th2) and IL-17A (Th17) were determined with canine-specific ELISA kits (DuoSet ELISA, R&D Systems; Minneapolis, WI, USA) following the manufacturer's instructions.

Statistical analyses

For between-group comparisons, the Kruskal–Wallis test was employed, whereas for paired analysis of parameters before and after intervention within groups the Wilcoxon signed rank test was used. Correlation between cytokine concentrations and both clinical scores and bacterial overgrowth were analysed with a Spearman correlation test (*p*). Statistical analyses were conducted with SPSS Statistics 24 software (IBM; Armonk, NY, USA). A *P*-value < 0.05 was considered significant.

Results

Changes in the cytokine secretion profile of allergen-stimulated PBMCs in the placebo and treatment group

Table 2. Bacterial scores of food allergic dogs pre- and post-treatment, receiving sublingual immunotherapy, by case.

Group	Case number	Bacterial score		
		Pre	Post	Difference
P	1	4	3	1
P	2	4	4	0
P	3	2	3	-1
P	7	2	2	0
P	10	3	3	0
P	12	3	2	1
T	4	2	1	1
T	6	3	0	3
T	8	2	2	0
T	9	3	1	2
T	11	2	0	2

Results before and after therapy and their differences. The presence of bacteria and number of bacteria engulfed by neutrophils was evaluated using a 0–4 severity scale (0, none seen; 1, < 1/HPF; 2, 1–5/HPF; 3, 5–10/HPF; 4, > 10/HPF).

P, placebo; T, treatment.

are summarized in Table 1, whereas the data for individual dogs are presented in Figure 1. IL-10 was undetectable in samples from all dogs at the beginning of the study and increased significantly after FA-SLIT in four dogs' samples. The within-group IFN-γ concentration was increased significantly by FA-SLIT (as compared to baseline), but not by placebo treatment. However, there was no statistical difference between the two groups at the end of the study. IL-4 could be detected from several samples in each group prior to treatment, but no significant changes were detected within or between groups.

At the start of the study, IL-17A was detectable in all T group and four of six P group samples. IL-17A concentrations decreased significantly after the experiment in both groups (placebo: *P* < 0.05; treatment: *P* < 0.01), but the effect was more pronounced in the treatment group as compared to the placebo group (*P* < 0.05). IL-17A levels were inversely correlated with IFN-γ at the beginning (*p* -0.61; *P* < 0.05), but not at the end of the study.

A clear decrease in bacterial score was seen in four of five treated dogs (Table 2). The difference between pre- and post-treatment bacterial scores was correlated with the difference between pre- and post-treatment IL-17A concentrations (*p* 0.84; *P* = 0.001) and the bacterial scores were inversely correlated with IL-10 concentrations post-treatment (*p* -0.66; *P* < 0.05). The latter was in parallel with the improvement of PVAS and CADESI scores. This was not seen in the placebo group (Table 3).

Discussion

To the best of the authors' knowledge, this is the first study to investigate the effect of immunotherapy for canine AFR and the associated cytokine expression of allergen-stimulated PBMCs. A significant increase in IL-10 was observed for dogs undergoing FA-SLIT. Human studies have led to variable results, where IL-10 increased after FA-SLIT, but not in all patients.¹ The results reported here reflect those observed in atopic dogs where ASIT induced increased IL-10 levels.⁸

Our results also suggest that FA-SLIT enhances IFN-γ concentrations without affecting IL-4 levels, which would be consistent with promotion of Th1 differentiation and a

Table 3. Individual pruritus Visual Analog Scale (PVAS) and Canine Atopic Dermatitis Extent and Severity Index, fourth iteration (CADESI-04) scores, and interleukin (IL)-10 and IL-17A concentrations, before (pre) and after (post) therapy and their differences (D) in dogs with adverse food reactions.

Group	Case no	PVAS			CADESI-04			IL-10 (pg/mL)			IL-17A (pg/mL)		
		Pre	Post	D	Pre	Post	D	Pre	Post	D	Pre	Post	D
P	1	7.2	7.1	0.1	51	46	5	0	0	-	24.4	0	24.4
P	2	8.7	7.5	1.2	57	56	1	0	0	-	31.94	19	12.94
P	3	6.1	6.2	-0.1	30	39	-9	0	0	-	19	0	19
P	7	6.2	6.5	-0.3	35	30	5	0	0	-	0	0	0
P	10	6.2	5.7	0.5	72	70	2	0	0	-	0	0	0
P	12	6.8	6.1	0.7	44	40	4	0	0	-	37	0	37
T	4	7.5	6.4	1.1	32	21	11	0	21.66	21.66	124.2	0	124.2
T	6	5.6	4	1.6	37	16	21	0	44	44	228.6	0	228.6
T	8	5.6	4	1.6	31	20	11	0	3	3	203.88	189.8	14.08
T	9	7.8	5.1	2.7	40	19	21	0	0	-	72.59	0	72.59
T	11	3.6	3.5	0.1	26	20	6	0	60.34	60.34	0	0	0

P, placebo group; T, treatment group.

shift to a Th1 dominant state. These findings are supported by previous human studies, although increased IFN- γ is not always a consistent feature.² A prior canine study reported higher IL-4 mRNA expression as compared to controls in both lesional and nonlesional skin of dogs with AFR; however, there were no differences in PBMCs.⁹ Interestingly, our findings mirror those of a previous study that showed an increase of IFN- γ and no changes in IL-4 in atopic dogs receiving ASIT.¹⁰

This is also the first report to describe IL-17A production in PBMCs of dogs with AFR. IL-17A producing cells have been demonstrated in healthy dogs and in dogs with leishmaniosis or naturally occurring chronic inflammatory diseases. In humans it has been demonstrated that IL-17A is involved in immune defence against bacterial infections and in the development of various immune-mediated diseases including psoriasis and allergic diseases (e.g. asthma, allergic rhinitis and atopy). Although we have shown that IL-17A levels were more profoundly decreased in treated dogs (versus placebo) at the end of the study, this was not correlated with the severity of clinical lesions nor with the intensity of pruritus (Table 3). This finding contradicts a previous report which argued that the percentage of Th17 cells was correlated with the severity of atopic eczema in people.¹¹ However, in agreement with that study, the IFN- γ and IL-17A levels were correlated in our canine subjects.

In addition, the decrease in IL-17A concentrations after FA-SLIT treatment was significantly correlated with a decrease in bacterial counts, suggesting that microbial stimuli may activate IL-17A secretion.¹² However, IL-10 levels were inversely correlated with bacterial counts. We therefore hypothesize that the increase in IL-10 levels suppressed inflammatory responses against culprit allergens which, in turn, decreased susceptibility to bacterial colonization. To prove this order of events it would be necessary to control bacterial colonization by treatment with antimicrobials and observe if the same change in IL-10 had occurred at the end of SLIT.

In conclusion, FA-SLIT appears to modulate the immune system where it is associated with a Th1 polarization and increased IL-10 levels, suggesting tolerance induction. IL-17A appears to be elevated in dogs with

AFR; however, future studies with more cases should be performed to understand if IL-17A has a role in the pathogenesis or development of AFR or acts against infections which are common in allergic dogs.

References

- Kim EH, Bird JA, Kulis M et al. Sublingual immunotherapy for peanut allergy: clinical and immunologic evidence of desensitization. *J Allergy Clin Immunol* 2011; 127: 640–646.
- Akdis CA, Blesken T, Akdis M et al. Role of interleukin 10 in specific immunotherapy. *J Clin Invest* 1998; 1: 98–106.
- Fuentes-Aparicio V, Alonso-Lebrero E, Zapatero L et al. Induction of Treg cells after oral immunotherapy in hen's egg-allergic children. *Pediatr Allergy Immunol* 2014; 25: 103–106.
- Maina E, Cox E. A double blind, randomized, placebo controlled trial of the efficacy, quality of life and safety of food allergen-specific sublingual immunotherapy in client owned dogs with adverse food reactions: a small pilot study. *Vet Dermatol* 2016; 27: 361–e91.
- Hill PB, Lau P, Rybnicek J. Development of an owner-assessed scale to measure the severity of pruritus in dogs. *Vet Dermatol* 2007; 18: 301–308.
- Olivry T, Saridomichelakis M, Nuttall T et al. Validation of the Canine Atopic Dermatitis Extent and Severity Index (CADESI)-4, a simplified severity scale for assessing skin lesions of atopic dermatitis in dogs. *Vet Dermatol* 2014; 25: 77–85.
- de Bruin T, de Rooster H, van Bree H et al. Lymphocyte proliferation to collagen type I in dogs. *J Vet Med A Physiol Pathol Clin Med* 2007; 54: 292–296.
- Keppel KE, Campbell KL, Zuckermann FA et al. Quantitation of canine regulatory T cell populations, serum interleukin-10 and allergen-specific IgE concentrations in healthy control dogs and canine atopic dermatitis patients receiving allergen-specific immunotherapy. *Vet Immunol Immunopathol* 2008; 123: 337–344.
- Veenhof EZ, Knol EF, Schlotter YM et al. Characterisation of T cell phenotypes, cytokines and transcription factors in the skin of dogs with cutaneous adverse food reactions. *Vet J* 2011; 187: 320–324.
- Shida M, Kadoya M, Park S-J et al. Allergen-specific immunotherapy induces Th1 shift in dogs with atopic dermatitis. *Vet Immunol Immunopathol* 2004; 102: 19–31.
- Koga C, Kabashima K, Shiraishi N et al. Possible pathogenic role of Th17 cells for atopic dermatitis. *J Invest Dermatol* 2008; 128: 2,625–2,630.
- Eyerich K, Pennino D, Scarponi C et al. IL-17 in atopic eczema: linking allergen-specific adaptive and microbial-triggered innate immune response. *J Allergy Clin Immunol* 2009; 123: 59–66.

Résumé

Contexte – L'immunothérapie spécifique d'allergène alimentaire (FA-SLIT) est considérée comme une approche nouvelle, sûre et efficace chez les chiens présentant des réactions alimentaires indésirables (AFR).

Hypothèses/Objectifs – Étudier les changements des cytokines associés à FA-SLIT.

Sujets – Onze chiens avec AFR confirmé.

Méthodes – Les participants ont reçu soit une dose croissante de FA-SLIT ou un placebo pendant six mois. Une provocation alimentaire orale a été réalisée au début et à la fin de l'étude ainsi que des examens cliniques et des prélèvements de cytologie bactérienne de surface et sanguine. Les cellules mononuclées sanguines périphériques ont été stimulées avec l'antigène alimentaire en cause. Les tests ELISA ont été utilisés pour quantifier les interleukines (IL)-10, IFN- γ , IL-4 et IL-17A dans le surnageant des cellules stimulées.

Résultats – Les taux de IL-10 et de IFN- γ étaient significativement augmentés à la fin de l'étude dans le groupe traitement (T), comparé au groupe placebo (P), tandis qu'aucun changement n'a été rencontré dans les taux de IL-4. Les taux de IL-17A étaient diminués dans les deux groupes (mais plus intensément dans le groupe T). Les scores bactériens sur la peau étaient positivement corrélés avec IL-17A avec les concentrations de IL-10. Les interleukines n'étaient pas corrélées avec les scores cliniques.

Conclusion et importance clinique – le FA-SLIT peut moduler la réponse allergique vers des phénotypes cellulaires TH1 et Treg et induire une tolérance chez les chiens avec AFR. Ainsi, FA-SLIT peut être un outil de désensibilisation des chiens avec AFR. Cependant, davantage de données sur un plus grand nombre de cas et un plus large panel de cytokines sont nécessaires pour corroborer les résultats et pour élucider le mécanisme d'action de réponse aux FA-SLIT par les chiens atteints d'AFR.

Resumen

Introducción – La inmunoterapia sublingual específica para alérgenos de los alimentos (FA-SLIT) se considera un método nuevo, seguro y eficaz en perros con reacciones adversas a los alimentos (AFR).

Hipótesis/Objetivos – Investigar los cambios en citoquinas clave asociadas con FA-SLIT.

Animales – Once perros con AFR confirmada.

Métodos – Los animales recibieron una dosis ascendente de FA-SLIT o placebo durante un período de seis meses. Se realizó una exposición alimenticia oral al inicio y al final del estudio, junto con exámenes clínicos y toma de muestras para citología bacteriana superficial y de sangre. Las células mononucleares de sangre periférica se estimularon con el antígeno alimentario problema. Se utilizaron métodos ELISA para cuantificar Interleuquina 10 (IL)-10, IFN- γ , IL-4 e IL-17A en el sobrenadante de las células estimuladas.

Resultados – Los niveles de IL-10 e IFN- γ aumentaron significativamente al final del estudio en el grupo de tratamiento (T), en comparación con el grupo placebo (P), mientras que no se encontraron cambios en los niveles de IL-4. Los niveles de IL-17A disminuyeron en ambos grupos (pero más profundamente en T). Los valores de poblaciones bacterianas en la piel se correlacionaron positivamente con IL-17A y se correlacionaron inversamente con las concentraciones de IL-10. Las interleuquinas no se correlacionaron con los valores clínicos.

Conclusión y importancia clínica – FA-SLIT puede modular la respuesta alérgica hacia fenotipo celular de Th1 y Treg, e inducir tolerancia en perros con AFR. Por lo tanto, FA-SLIT puede ser una opción de tratamiento para desensibilizar perros con AFR. Sin embargo, se necesitan más datos, un mayor número de animales y un panel más amplio de citoquinas para corroborar estos hallazgos, y para dilucidar el mecanismo de acción en las respuestas a FA-SLIT en perros con AFR.

Zusammenfassung

Hintergrund – Futterallergen-spezifische sublinguale Immuntherapie (FA-SLIT) wird als neue, sichere und effektive Herangehensweise bei Hunden mit Nebenwirkungen auf Futtermittel (AFR) betrachtet.

Hypothese/Ziele – Eine Untersuchung von Änderungen der Zytokine, welche bei FA-SLIT eine Rolle spielen.

Tiere – Elf Hunde mit bestätigter AFR.

Methoden – Die teilnehmenden Tiere erhielten entweder eine steigende Dosis von FA-SLIT oder Placebo über eine Zeitdauer von sechs Monaten. Es wurden orale Futtermittelprovokationen am Anfang und am Ende der Studie durchgeführt, gemeinsam mit klinischen Untersuchungen und der Entnahme bakterieller Zytologie der Haut und Blutuntersuchungen. Es wurden periphere Blutmononukleäre Zellen mit dem verursachenden Futterallergen stimuliert. Es wurden ELISA Methoden angewendet, um Interleukin (IL)-10, IFN- γ , IL-4 und IL-17A aus dem Überstand der stimulierten Zellen, zu quantifizieren.

Ergebnisse – IL-10 und IFN- γ Werte waren am Ende der Studie in der Behandlungsgruppe (T) im Vergleich zur Placebogruppe (P) signifikant erhöht, während bei den IL-4 Werten keine Veränderungen gefunden wurden. IL-17A Werte waren in beiden Gruppen erniedrigt (allerdings deutlicher in der T Gruppe). Die Auswertung der Bakterien auf der Haut war mit IL-17A positiv korreliert sowie invers korreliert mit IL-10 Konzentrationen. Die Interleukine waren mit den klinischen Werten nicht korreliert.

Schlussfolgerungen und klinische Bedeutung – FA-SLIT kann die allergische Antwort in Richtung Th1 und Treg Zell Phänotypen regulieren, somit eine Toleranz bei Hunden mit AFR induzieren. Folglich könnte die FA-SLIT eine Möglichkeit sein, Hunde mit AFR zu desensibilisieren. Allerdings sind weitere Daten von einer größeren Anzahl an Fällen und ein breiteres Spektrum an Zytokinen nötig, um diese Ergebnisse zu stärken und um die Wirkungsmechanismen der FA-SLIT bei Hunden mit AFR zu beleuchten.

要約

背景 – 食物アレルギー特異的舌下免疫療法(FA-SLIT)は、食物有害反応(AFR)を有するイヌにおいて、安全かつ有効な新しいアプローチであると考えられている

仮説/目的 – FA-SLITに関連する主要サイトカインの変化を調べること。

供与動物 – AFRと診断された犬11頭。

方法 – 患者は、FA-SLITまたはプラセボの漸増投与を6ヶ月間にわたって受けた。試験の開始時および終了時に、身体検査、皮膚表面の細菌細胞診検査および血液採取を実施し、加えて経口食物負荷試験も行った。末梢血単核球を原因食物抗原で刺激した。ELISA法を用いて、刺激された細胞の上清中のインターロイキン(IL)-10、IFN- γ 、IL-4およびIL-17Aを定量した。

結果 – プラセボ群(P)と比較して、治療群(T)における試験終了時のIL-10およびIFN- γ レベルは有意に増加したが、IL-4レベルに変化は見られなかった。IL-17Aレベルは両群で減少した(ただし、Tにおいてより減

少した)。皮膚の細菌スコアはIL-17Aと正の相関を示し、IL-10濃度と逆相関した。インターロイキン値は臨床スコアと相関しなかった。

結論および臨床的な重要性 – FA-SLITは、アレルギー反応をTh1およびTregに傾け、AFRを有するイヌにおける耐性を誘導し得る。したがって、FA-SLITは、AFR犬を脱感作するための手段となりえる可能性がある。しかしながら、これらの知見を裏付け、AFRを有するイヌに対するFA-SLITの作用機序を解明するためには、より多くの症例のより広範なサイトカインパネルが必要である。

摘要

背景 – 食物過敏原特異性舌下免疫治療(FA-SLIT)被认为是食物不良反应患犬的一种新的、安全且有效的治疗方法。

假设/目的 – 调查与FA-SLIT相关的关键性细胞因子的变化。

动物 – 十一只确认患有AFR的犬。

方法 – 参与者在六个月内接受剂量递增的FA-SLIT,或者安慰剂。在本研究开始和结束时,均进行经口食物激发,同时配合临床检查,并进行皮肤表面细菌细胞学和血液检查。用致敏的食物抗原刺激外周血单核细胞。使用ELISA方法对刺激细胞的上清液中白细胞介素(IL)-10,IFN- γ ,IL-4和IL-17A进行定量分析。

结果 – 与安慰剂组(P)相比,治疗组(T)在研究结束时的IL-10和IFN- γ 水平显著升高,然而IL-4水平没有变化。两组IL-17A水平均降低(但T组更显著)。皮肤细菌评分与IL-17A呈正相关,与IL-10浓度呈负相关。白细胞介素与临床评分无关。

结论和临床意义 – FA-SLIT可能调节针对Th1和Treg细胞表型的过敏反应,并诱导AFR患犬产生耐受性。因此,FA-SLIT可能是使AFR患犬脱敏的一种途径。然而,需要更多病例和更广泛细胞因子的数据来证实这些发现,以阐明AFR患犬对FA-SLIT的反应机制。

Resumo

Contexto – A imunoterapia sublingual para alérgenos alimentares (FA-SLIT) é uma nova abordagem terapêutica nova que parece ser segura e efetiva no tratamento de cães com reações adversas a alimentos (AFR).

Hipótese/Objetivos – Investigar as alterações nas principais citocinas associadas com FA-SLIT.

Animais – Onze cães com AFR confirmada.

Métodos – Os participantes receberam doses crescentes de FA-SLIT ou placebo durante seis meses. Desafio com alimentos por via oral foi realizado no início e no fim do estudo, em conjunto com exames clínicos e coleta de citologia bacteriana na superfície cutânea e de sangue. As células mononucleares de sangue periférico foram estimuladas com alérgenos alimentares. O método de ELISA foi utilizado para quantificar a concentração de interleucina (IL) 10, IFN- γ , IL-4 e IL-17A no sobrenadante das células estimuladas.

Resultados – Os níveis de IL-10 e IFN- γ estavam significativamente elevados no final do estudo no grupo de tratamento (T), comparado com o grupo placebo (P), enquanto nos níveis de IL-4 não foram observadas alterações. Os níveis de IL-17A estavam reduzidos em ambos os grupos (mas mais intensamente no T). Os escores de quantidade de bactérias na pele correlacionaram positivamente com a concentração de IL-17A e inversamente com a de IL-10. Não houve correlação das interleucinas com os escores clínicos.

Conclusão e importância clínica – A FA-SLIT pode modular a resposta imune alérgica no sentido dos fenótipos celulares Th1 e Treg, e induzir tolerância nos cães com AFR. Desta forma, FA-SLIT pode ser uma maneira de dessensibilizar cães com AFR. Entretanto, mais dados de estudos com maior número de casos e um painel mais amplo de citocinas são necessários para confirmar estes resultados e elucidar o mecanismo de ação para as respostas de FA-SLIT em cães com AFR.