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Effects of polyunsaturated fatty acids on isolated canine peripheral blood mononuclear cells and cytokine expression (IL-4, IFN-γ, TGF-β) in healthy and atopic dogs

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Abstract

Polyunsaturated fatty acids (PUFA) have been used to treat dogs with atopic dermatitis but the mechanism of action has not been well understood. The aim of this study was to evaluate the *in vitro* influence of PUFA on canine peripheral blood mononuclear cells (PBMC). PBMC isolated from eleven dogs with atopic dermatitis and eleven healthy control dogs were stimulated with concanavalin A and *Dermatophagoides farinae* extract in the presence of linoleic acid (LA), γ-linolenic acid (GLA), α-linolenic acid (ALA), eicosapentaenoic acid (EPA)/docosahexaenoic acid (DHA) and GLA/EPA/DHA. Subsequently, quantitative polymerase chain reaction (qPCR) for interferon (IFN)-γ, interleukin (IL)-4 and transforming growth factor (TGF)-β m-RNA was performed. In the presence of concanavalin A, only PBMC of healthy dogs showed a gradual reduction in proliferation index from incubation without PUFA to incubation with ALA, EPA/DHA and GLA/EPA/DHA, respectively. A similar reduction was seen in normal and in atopic dogs in the presence of *D. farinae* allergen after incubation with ALA, EPA/DHA and GLA/EPA/DHA. In both groups IL-4 and IFN-γ but not TGF-β gene transcription was upregulated, when cells were incubated with *D. farinae*. Allergen-induced upregulation was not influenced by incubation with PUFA. These findings suggest that PUFA are able to influence proliferation of peripheral blood mononuclear cells in healthy and atopic dogs but do not seem to influence gene transcription of IL-4, IFN-γ and TGF-β.

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Introduction

Atopic dermatitis (AD) is a common diagnosis in small animal practice[1] frequently associated with IgE antibodies to environmental allergens.[2] Canine atopic dermatitis (CAD) is a genetically predisposed inflammatory and pruritic allergic skin disease with similar immunological changes and clinical signs to those of human atopic dermatitis and consequently has been proposed as a suitable model of the human condition.[3,4] Clinically, the predominant sign of AD in both humans and dogs is pruritus. Symptomatic treatment modalities for canine atopic dermatitis are predominantly systemic or topical glucocorticoids,[5] calcineurin inhibitors,[5] antihistamines,[5] fatty acid supplementation,[6] and topical therapies.[5] Adverse effects to glucocorticoid therapy are common,[7] and recently more attention has been paid to comparatively safe treatments such as antihistamines[5,8] and polyunsaturated fatty acid (PUFA) supplementation[6,9] despite their lower success in reduction of pruritus.

PUFA can modulate the inflammatory response[10,11] and oral fatty acid supplementation has been shown to benefit the inflammation and pruritus associated with CAD.[12] In a double-blinded, placebo-controlled randomized study, 10–20% of dogs with atopic dermatitis were in complete remission and 40% were significantly better after supplementation with PUFA.[13] Essential fatty acids have been used for the treatment of canine atopic dermatitis since 1987 and can be divided into the two main groups of omega 3 and omega 6 fatty acids. The most important omega 3 fatty acids are α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The most important omega 6 fatty acids are γ-linoleic acid (GLA) and linoleic acid (LA). However, which of these groups or which ratio is best suited for the treatment of canine atopic dermatitis is still unclear.[6,9,12] Similarly, the molecular mechanisms of PUFA in atopic

dermatitis have not been elucidated but modulation of eicosanoid production, correction of epidermal lipid defects and inhibition of cellular activation and cytokine secretion are all possibilities.[2]

Langerhans cells with surface-bound IgE are present in atopic skin[14] and are thought to activate T_H2 cells.[3] T_H2 cytokines (IL-4, IL-5, IL-6, IL-13) produced by these T_H2-type cells induce isotype switching in B cells that subsequently produce IgE. In addition, these cytokines stimulate macrophages and eosinophils to produce mediators such as IL-12.[15] This leads to activation of T_H1-type cells and the presence of IFN-γ producing T cells correlates with chronicity and severity of AD skin lesions.[3,15] IFN-γ is a T_H1-type cytokine which promotes cell-mediated immunity. Thus, the presence of T_H2-type cytokines, most notably IL-4, is characteristic for acute lesions.[15]

The purpose of this study was to assess the *in vitro* effect of various PUFA on the proliferative response of peripheral blood mononuclear cells (PBMC) and to investigate the influence of PUFA on the expression of IL-4, IFN-γ and TGF- β by PBMC from healthy and atopic dogs. IFN-γ and IL-4 were chosen as representatives for the T_H1 and T_H2 response, respectively.

Methods

Subjects

Atopic dermatitis was diagnosed in dogs ($n = 21$) by history, clinical examination and exclusion of differential diagnoses such as adverse reactions to food or scabies with appropriate tests or treatments as previously described.[16] All dogs fulfilled Willemse's criteria for the diagnosis of atopic dermatitis[17] and each was sensitive to *Dermatophagoides farinae* as determined by skin or serum testing for allergen-specific IgE. Healthy dogs ($n = 21$) without reported or clinical evidence of disease owned by students and staff of the Veterinary Medical Teaching Hospital/University of Munich were used as controls.

PBMC preparation

Heparinized blood was collected from the jugular vein of eleven atopic and eleven normal dogs and immediately layered on top of an equal volume of Ficoll (density 1077 mg/cm^3, Biocoll Separating Solution, Biochrom AG, Berlin, Germany). Following density centrifugation at room temperature for twelve minutes at $600 \times g$, the interphase was collected and washed twice with ice cold phosphate buffered saline. The cell pellet was suspended in 1 mL of RPMI 1640 supplemented with glutamine, 1% penicillin/streptomycin and 10% fetal calf serum (Biochrom AG, Berlin, Germany). The viability of the cell preparation was determined using trypan blue and the cell density was adjusted to 5×10^6 cells/mL.

Proliferation assays

PBMC were isolated and incubated without and with various fatty acids. The fatty acids used were linoleic acid (LA), γ-linoleic acid (GLA), α-linolenic acid (ALA), eicosapentaenoic acid and docosahexaenoic acid (EPA/DHA) and a combination of GLA and EPA/DHA (ratio 1 : 2). The fatty acids were dissolved in dimethyl sulfoxide (DMSO) and used at a concentration of 10^{-5} M which was shown in preliminary studies to have no influence on the proliferation of PBMC in negative controls or when incubated with *D. farinae* antigen. All the fatty acids were tested by Sigma (Sigma-Aldrich, Buchs, Switzerland) for purity (between 90 and 98%) and capacity to dissolve. Cells were incubated without additional antigen (negative control), 50 μg/mL of the specific *D. farinae* extract (1 : 100 weight per volume, 40.000 PNU/mL, Greer Laboratories, Lenoir, NC, USA, referred to as allergen) or concanavalin A (positive control, 10 μg/mL, Amersham Pharmacia Biotech, Freiburg, Germany); preliminary studies showed a more potent PBMC stimulation with concanavalin A in comparison to phytohemagglutinin (Sigma-Aldrich, Buchs, Switzerland), and established the effectiveness of the selected concentration of the *D. farinae* extract.[18] PBMC were stimulated in 96-well plates. The proliferation assay was performed in triplicate. The allergen, negative control (standard medium) and positive control (concanavalin A) were diluted using cell culture medium (RPMI 1640 supplemented with glutamine, 1% penicillin/streptomycin and 10% foetal calf serum). Fifty microlitres of this dilution was placed in three wells of a 96 well flat bottom plate for each dog and 50 μL of the different essential fatty acids (LA, GLA, ALA, EPA/DHA and GLA/EPA/DHA) at a concentration of 10^{-5} M were added. Finally 100 μL of the cell suspension at a concentration of 5×10^6 cells/mL was added to all wells. The plates were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 3 days. Thereafter, 20 μL ^{3}H-thymidine (Amersham Pharmacia Biotech, Freiburg, Germany) in a final concentration of 1 μCi was added 18 h before the end of the assay to each well. Cells were harvested on filters using a cell harvester, and the incorporation of radioactivity was determined on a Microplate Scintillation Counter (Canberra; Packard, Dreieich, Germany). Data are presented as stimulation index defined as cpm of stimulated sample/cpm of unstimulated control.

Cytokine expression

In the second part of the study, PBMC were isolated from 10 atopic dogs and ten healthy dogs as described above and in each instance 1 mL of the cell suspension at a concentration of 2×10^7 cells/mL was placed in three wells of a 24 well flat bottom plate. The PBMC were stimulated for 6 h at 37 °C in a humidified atmosphere with 5% CO_2, because preliminary studies showed a higher cytokine expression than after 10 and 18 h (data not shown). Because of the high sensitivity of cytokine expression the PBMC were incubated with 3.1 μL DMSO solution as a negative control; they were also incubated with allergen dissolved in DMSO (1 : 1000), and with allergen, DMSO and LA, EPA/DHA or GLA/EPA/DHA. Fatty acids were used in a concentration of 10^{-5} M. After stimulation RNA isolation was performed using peqGOLD TriFast™ Solution (peqlab, Erlangen, Germany) according to the manufacturer's instructions and RNA was dissolved in 15 μL RNAse free water. RNA quality was checked using an Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). RNA Integrity Numbers (RIN) were always above 7.5. For quantification RNA samples were measured using an Eppendorf biophotometer (Eppendorf, Hamburg, Germany). To avoid genomic DNA contamination and to transcribe RNA in cDNA the QuantiTect® Reverse Transkription Kit (Qiagen, Hilden, Germany) was used.

Quantification of cytokine expression was performed by qPCR using SYBR green technology (QuantiTect® SYBR Green PCR Kit; Qiagen, Hilden, Germany). For each cytokine, specific primer pairs were synthesized based on the available cytokine sequences (Table 1). All primers had similar annealing temperatures (59 °C) and were quality checked with Primer express 3.0 to analyze secondary structures. The primer pairs were evaluated by a NCBI-BLAST (http://www.ncbi.nlm.nih.gov) search to minimize the chance of unspecific binding and were quality checked by amplification of test cDNA by polymerase chain reaction. The products of the polymerase chain reaction were evaluated by agarose gel electrophoresis to confirm that there was only one specific amplification. Other bands in agarose gel electrophoresis would demonstrate an unspecific amplification due to poor primers or contained cDNA. To evaluate the primer pairs in the qPCR, a standard curve was created using a Log_{10} dilution series over seven magnitudes in combination with a dissociation curve analysis. Finally the sequences of the PCR products were determined at the GATC (Konstanz, Germany) and checked in NCBI-Blast before use in the main testing. This was important to evaluate that only the gene of interest was amplified. The results of the expression of IFN-γ, IL-4 and TGF-β were normalized to two house keeping genes (β-actin and 18S rRNA). To quantify the gene

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Table 1. Specifically designed primers for selected cytokines, their sequence, length, efficiency and annealing temperature

Gene	Sequence	Sequence of PCR Product	Length of basepairs	Efficiency	Annealing temperature (°C)
IL-4	TTCCAACTTCAAGGCAATTAACC	atcaTCAAAatgtTGAACATCCTCACAGCG	91	1907	59
	CGTTTCTCGCTGTGAGGATGT	AGAAACG			59
IFN-γ	GCTTTGCGTGATTTTGTGTTCTT	GaaatagaaaaCCTAAAGGAATATTTTAATG	113	1848	59
	CACCGTCCGATACATCTGGATTA	CAAGTAATCCAGATGTATCGGACGGtg			59
TGF-β	GTATATGGCCCCCGAAGTTCTAG	cGtgCCGACATCTATGcaATGGGCTTAGT	98	1876	59
	CCCAGAATACTAAGCCCATTGC	ATTCTGGGa			59

expression changes induced in the different treatment groups of the cell culture experiment the $\Delta\Delta C_t$ relative quantification method was used.

Statistical analysis

The proliferation indices were compared with a Friedman test and Dunn's multiple comparison test and a $P < 0.05$ was considered significant. These tests were also used to compare the influence of PUFA on cytokine production within each group. Cytokine gene transcriptions in atopic and healthy dogs were compared with a Kruskal–Wallis test and Dunn post test and a $P < 0.05$ was again considered significant. Analysis was performed with the Graphpad Prism and Instat software (Version 4.0 and 3.06 respectively; Graphpad Software, San Diego, CA, USA).

Results

Proliferation assays

In the presence of concanavalin A, PBMC from healthy dogs showed a gradual reduction in the proliferation index when incubated with LA, GLA, ALA, EPA/DHA and GLA/EPA/DHA respectively in comparison to incubation without PUFA (Figure 1). This difference was statistically significant between the control and ALA, EPA/DHA or GLA/EPA/DHA incubations respectively ($P < 0.05$). A similar reduction was seen in the presence of allergen where all PUFA incubations except with LA led to a significant reduction of proliferation ($P < 0.001$) (Figure 2). In contrast, the atopic dogs showed a similar reduction only when stimulated by allergen (Figure 3). This reduction was significant after incubation with ALA ($P < 0.001$), EPA/DHA and GLA/EPA/DHA ($P < 0.01$ for both combinations).

Cytokine expression

Compared to the negative control, there was a marked upregulation of IL-4 (Figure 4) and IFN-γ in PBMC from normal and atopic dogs, when incubated with allergen. Allergen-induced upregulation was not reversed by incubation with PUFA. TGF-β expression was not significantly altered in PBMC of atopic dogs, but it was downregulated in healthy dogs under the influence of EPA/DHA (data not shown).

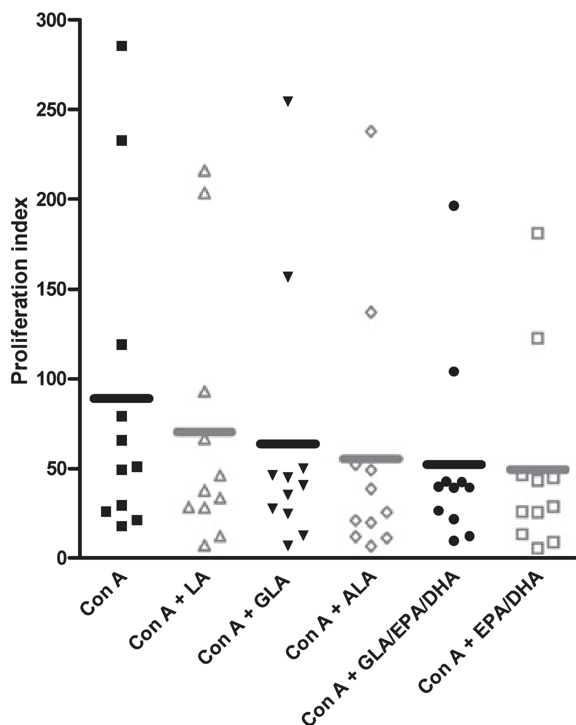

Figure 1. Healthy dogs showed a gradual reduction in the proliferation index from incubation with LA, GLA, ALA, EPA/DHA and GLA/EPA/DHA respectively in the presence of concanavalin A, compared with incubation without PUFA (Bars represent mean values). This difference was statistically significant between the control and ALA, EPA/DHA, GLA/EPA/DHA incubations ($P < 0.05$).

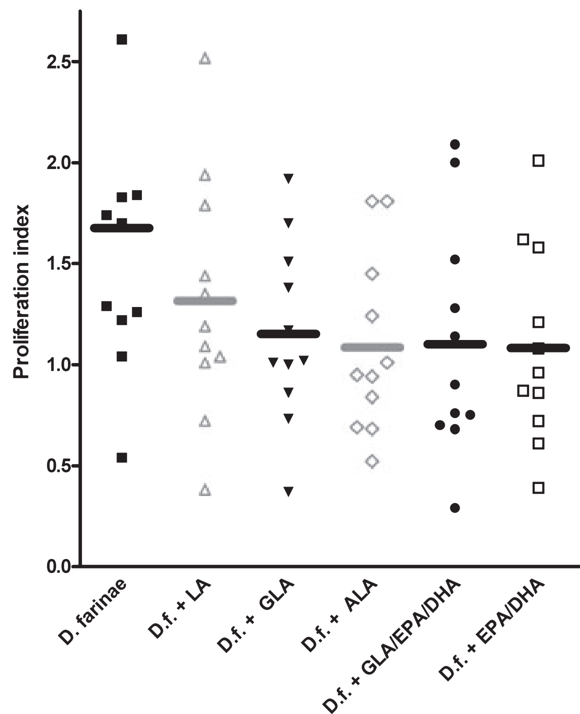

Figure 2. A significant reduction in PBMC proliferation ($P < 0.001$) occurred in the presence of allergen (*D. farinae*) in healthy dogs with all PUFA except LA in comparison to the control incubation (Bars represent mean values).

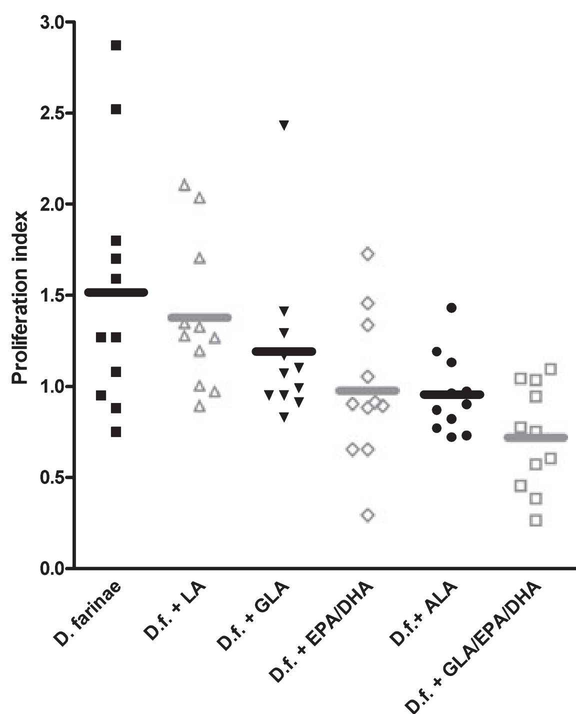

Figure 3. A reduction in PBMC proliferation occurred in atopic dogs when incubated with ALA ($P < 0.001$), EPA/DHA ($P < 0.01$) and GLA/EPA/DHA ($P < 0.01$) in the presence of allergen (*D. farinae*) (Bars represent mean values).

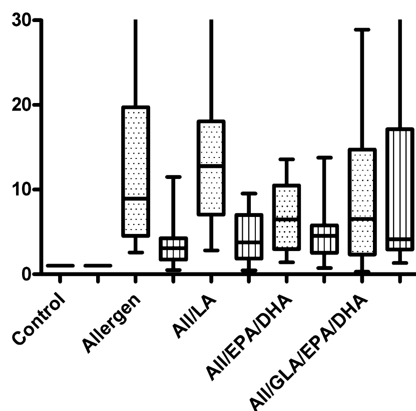

Figure 4. IL-4 expression of PBMC in healthy (dotted boxes) and atopic dogs (striped boxes) after incubation with allergen and various PUFA. Although there was an increase in IL-4 expression after incubation with the allergen (All) *D. farinae*, adding linoleic acid (LA), eicosapentaenoic acid/docosahexaenoic acid (EPA/DHA) or gamma-linoleic acid (GLA)/EPA/DHA did not significantly change the IL-4 expression. The boxes represent 50% of the values, the lines represent the median value.

Discussion

These results demonstrate that incubation of PBMC from normal dogs and dogs with atopic dermatitis with various PUFA and *D. farinae* extract led to a significant decrease of proliferation indices, but PUFA did not influence the cytokine gene transcription of IL-4, IFN-γ and TGF-β.

Although PUFA supplementation is not as effective as glucocorticoid therapy in the treatment of atopic dermatitis, it has been shown to be an alternative for some dogs with AD.[6] Proposed molecular mechanisms include modulation of eicosanoid production, inhibition of cellular activation and an increase in epidermal barrier function.[9] EPA and DHGLA both compete with arachidonic acid as a substrate for 5-lipoxygenase and cyclo-oxygenase, which converts these fatty acids to anti-inflammatory leukotrienes and prostaglandins.[9] Increases in anti-inflammatory eicosanoids and concurrent decreases in their proinflammatory counterparts presumably decrease cutaneous inflammation. However, leukotriene inhibitors are not very effective in treating CAD and are not recommended as therapy for this disease.[5] Thus, it seems unlikely that the mechanism of action of PUFA in CAD is due to an influence on leukotrienes.

The influence of PUFA on the immune response is increasingly recognised in human medicine and the results indicate that PUFA also influence T cell proliferation in the dog. This may contribute to the clinical effects of PUFA supplementation. The significantly decreased PBMC proliferation indices following incubation with PUFA and *D. farinae* extract in normal dogs with all PUFA except LA and in atopic dogs with all PUFA except LA and GLA, n-3 PUFA may be more effective in inhibiting PBMC proliferation than n-6 PUFA. In human patients, long-chain n-3 PUFA are incorporated into cell membranes, resulting in decreased production of pro-inflammatory eicosanoids and cytokines. When PBMC were stimulated with concanavalin A, PUFA were not able to decrease proliferation in atopic dogs. In these dogs, allergen-specific T cells are already activated by the disease process and it is possible that additional strong stimulation with concanavalin A proved too powerful to be suppressed by fatty acids.

It is difficult to compare the concentrations of fatty acids *in vitro* in this study with those used *in vivo*. However, based on previous studies evaluating polyunsaturated fatty acid concentrations in skin and serum,[13] the concentrations used in this *in vitro* study are lower than those achieved *in vivo* in the skin, but higher than in the serum. Skin and serum concentrations do not necessarily correlate well in atopic dogs. Mueller *et al.* determined PUFA concentrations in plasma and tissue before and after n-3 fatty acid supplementation in dogs with CAD[13] and found that the PUFA concentrations in skin or plasma were not correlated to the severity of the clinical signs or treatment response suggesting that efficacy depends on more than PUFA dose or ratio. Unidentified individual idiosyncratic factors may be relevant for individual responses.[13] Penetration of allergens into the skin contributes to the skin reactions.[19] Concentrations of cytokines and PBMC in local tissue may play a more important role in influencing allergic inflammation and need to be further evaluated.

A dietary ratio of n-3 : n-6 PUFA of 1 : 5 is frequently recommended in veterinary dermatology but is based on one study where the most prominent inhibition of LTB_4 release from neutrophils of beagles fed with diets containing various ratios of n-3 : n-6 fatty acids (1 : 5, 1 : 10, 1 : 20) occurred at a ratio of 1 : 5.[20] However, other ratios such as 1 : 2 or 1 : 1 were not evaluated. In a more recent study evaluating dietary management of canine atopic dermatitis, the diet with the most beneficial effect

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had an n-3 : n-6 ratio of 1 : 2.7.[21] In the present study, a 2 : 1 ratio of n-3 : n-6 PUFA chosen in the mixed group (GLA/EPA/DHA) and led to the most pronounced suppression of PBMC proliferation in atopic dogs after incubation with antigen.

Studies in humans showed inconsistent results regarding the influence of PUFA on the immune response. Gallai *et al*. reported decreased IFN-γ production by concanavalin A-stimulated blood lymphocytes after fish oil supplementation.[22] Wallace *et al.* described the effects of dietary fatty acids on the production of cytokines and their mRNA and suggested that PUFA can alter the T_H1-/T_H2-type cytokine balance.[23] Fish oil (n-3 PUFA) appears to be particularly potent at skewing this balance away from T_H1 toward T_H2.[24] In another study IL-4 release was unaltered after fish oil supplementation, whereas IL-10, IFN-γ and tumour necrosis factor (TNF)-α concentrations were increased. These results are in contrast to those described by others using EPA-rich fish oil supplementation. Changes in the proportion of DHA and EPA (3 : 1) exert different effects on neutrophil, monocyte and lymphocyte function, which may be a result of specific changes in gene expression.[25] In this study, an upregulation of IL-4 and IFN-γ, was seen when cells from normal and atopic dogs were incubated with allergen and not decreased when PUFA were added. Regulatory T cells are thought to play an important role in immune regulation.[26] In humans, they typically express CD4/CD25, although induced regulatory T cells may lack CD25.[26] These regulatory T cells are characterised by secretion of regulatory cytokines, particularly IL-10 and TGF-β. This latter cytokine was selected to evaluate an influence of PUFA on the induction of regulatory T cells and upregulation of the regulatory cytokine TGF-β. No such influence was detected in this current study. However, in contrast to IFN-γ and IL-4, TGF-β expression was not upregulated when cells were incubated with allergen; there was no effect of PUFA or allergen on the expression of TGF-β in this study.

In summary the results show an influence of PUFA on PBMC proliferation in the canine which may contribute to the clinical effects of PUFA supplementation. Incubation of PBMC with PUFA had no influence on the expression of IL-4, IFN-γ or TGF-β.

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Résumé Les acides gras polyinsaturés (PUFA) ont été utilisés pour traiter les chiens atteints de dermatite atopique mais leur mécanisme d'action reste mal compris. L'objectif de cette étude était d'évaluer l'influence *in vitro* des PUFA sur les cellules mononucléées (PBMC) du sang périphérique canin. Les PBMC isolées de onze chiens atopiques et onze chiens sains contrôles ont été stimulés avec la concanavalin A et un extrait de *Dermatophagoides farinae* en présence d'acide linoleique (LA), d'acide γ-linolenique (GLA), d'acide α-linolenique (ALA), d'acide eicosapentaenoique (EPA)/acide docosahexaenoique (DHA) and GLA/EPA/DHA. Par la suite, une PCR quantitative (Polymerase Chain Reaction) a été réalisée pour l'interféron (IFN)-γ, interleukin (IL)-4, et le transforming growth factor (TGF)-β m-RNA. En présence de concanavalin A, seuls les PBMC des chiens sains ont montrés une diminution graduelle de l'index de prolifération depuis l'incubation sans PUFA à l'incubation avec ALA, EPA/DHA et GLA/EPA/DHA respectivement. Une réduction similaire a été observée chez les chiens normaux et atopiques en présence de l'allergène *D. farinae* après incubation avec ALA, EPA/DHA and GLA/EPA/DHA. Dans les deux groupes IL-4 et IFN-γ mais pas dans le groupe TGF- β, la transcription a été régulée à la hausse lorsque les cellules étaient incubées avec *D. farinae*. Cette régulation induite par l'allergène n'a pas été influencée par l'incubation avec les PUFA. Ces résultats suggèrent que les PUFA peuvent influencer la prolifération des cellules mononucléées du sang périphérique chez les chiens sains et les chiens atopiques mais ne semblent pas influencer la transcription des gènes de IL-4, IFN-γ et TGF-β.

Resumen Los ácidos grasos poliinsaturados (PUFA) han sido utilizados en el tratamiento de perros con dermatitis atópica, pero el mecanismo de acción no ha sido bien elucidado. El propósito de este estudio fue evaluar la influencia *in vitro* de PUFA en células mononucleares caninas de sangre periférica (PBMC). PBMC se aislaron de once perros con dermatitis atópica y de once perros sanos como control. Estos se estimularon con concanavalina A y extracto de *Dermatophagoides faringe* en presencia de acido linoleico (LA), acido γ-linoleico (GLA), acido α-linoelico (ALA), ácido eicosapentanoico (EPA)/ácido docosahexanoico (DHA) y GLA/EPA/DHA. Posteriormente se realizó una reacción de polimerasa en cadena cuantitativa para el RNA mensajero de IFN- γ, IL-4 y factor transformador de crecimiento (TGF-β). En presencia de concanavalina A, sólo los PBMC de perros sanos mostraron una reducción gradual del índice proliferativo de la incubación sin PUFA a la incubación con ALA, EPA/DHA y GLA/EPA/DHA, respectivamente. Una reducción similar se observo en perros normales y atópicos en presencia del extracto de *D. farinae* tras la incubacion con ALA, EPA/DHA y GLA/EPA/DHA. En ambos grupos la transcripción de los genes de IL-4 e IFN- γ, pero no TGF- β estaba aumentada cuando las células se incubaron con *D. farinae.* La elevación de la transcripción inducida por el alergeno no estuvo afectada por la incubación con PUFA. Estos hallazgos indican que PUFA son capaces de afectar la proliferación de celulas mononucleares de sangre periférica en perros sanos y atópicos pero no parece afectar las transcripcion de los genes de IL-4, IFN- γ y TGF- β.

Zusammenfassung Mehrfach-ungesättigte Fettsäuren (PUFA) werden verwendet, um Hunde mit atopischer Dermatitis zu behandeln, wobei aber der Aktionsmechanismus nicht sehr gut verstanden wird. Das Ziel dieser Studie war es, den *in vitro* Einfluss von PUFA auf canine periphere mononukleäre Blutzellen (PBMC) zu evaluieren. PBMC, die von elf Hunden mit atopischer Dermatitis und von elf gesunden Hunden als Kontrollen isoliert worden waren, wurden mit Concanavalin A und *Dermatophagoides farinae* Extrakt im Beisein von Linolsäure (LA), γ-Linolsäure (GLA), α-Linolsäure (ALA), Eicosapentaensäure (EPA)/Docosahexaensäure (DHA) und GLA/EPA/DHA stimuliert. In der Folge wurde eine quantitative Polymerasekettenreaktion (qPCR) für Interferon (IFN)-γ, Interleukin (IL)-4 und Transforming Growth Faktor(TGF)-β mRNA durchgeführt. Mit Concanavalin A zeigten nur die PBMC der gesunden Hunde von der Inkubation ohne PUFA bis zur Inkubation mit ALA bzw. EPA/DHA bzw. GLA/EPA/DHA. eine graduelle Reduktion des Proliferationsindex. Eine ähnliche Reduktion wurde bei normalen und atopischen Hunden im Beisein von *D. farinae* Allergen nach der Inkubation mit ALA, EPA/DHA und GLA/EPA/DHA gefunden. In beiden Gruppen war die IL-4 und die IFN-γ, aber nicht die TGF-β Gen Transkription erhöht, wenn die Zellen mit *D. farinae* inkubiert wurden. Die Allergen-induzierte Erhöhung wurde von einer Inkubation mit PUFA nicht beeinflusst. Diese Ergebnisse weisen darauf hin, dass PUFA die Proliferation der peripheren mononukleären Blutzellen bei gesunden und atopischen Hunden beeinflussen können, dass sie aber die Gen Transkription von IL-4, IFN-γ und TGF-β scheinbar nicht beeinflussen.

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