

Atopic dermatitis in West Highland white terriers – Part III: early life peripheral blood regulatory T cells are reduced in atopic dermatitis

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Background – Regulatory T (Treg) cells are involved in homeostasis of immune regulation and suppression of inflammation and T-cell polarisation. Current knowledge regarding the role of Treg cells in the initiation of allergic disease is limited for both people and dogs.

Objectives – To explore the role of circulating Treg cells and their possible influencing factors, on the development of atopic dermatitis (AD).

Methods and materials – This study followed part of a birth cohort of West Highland white terrier dogs and classified them according to eventual clinical signs of AD (i.e. allergic versus healthy). The Treg phenotypes were assessed longitudinally by flow cytometry at 3, 3–12 and 12–36 months of age, and associated with development of AD. Different early life antigenic factors [endotoxins and allergens in house dust, *Toxocara canis*-specific immunoglobulin (Ig)E/IgG, allergen-specific and total IgE, skin microbiota] were measured at three months of age, and a possible association with Treg cell levels was assessed.

Results – The percentages of CD4⁺CD25⁺Foxp3⁺ Treg cells in healthy dogs were significantly higher at in 3-month-old (mean 4.5% healthy versus 3.3% allergic; $P = 0.021$) and <1-year-old (4.0% healthy versus 2.9% allergic; $P = 0.028$) dogs when compared to percentages of Treg cells in dogs that developed AD. There was a significantly positive correlation between the relative abundance of *Lachnospiraceae* on the skin and CD4⁺CD25⁺Foxp3⁺ Treg cells in puppies that became allergic ($r = 0.568$, $P = 0.017$).

Conclusion and clinical importance – Further large-scale studies are needed to identify the practical value of these findings in AD diagnosis, treatment and prevention.

Introduction

Atopic dermatitis (AD) is the most common chronic inflammatory skin disease in both people and dogs, substantially affecting quality of life with a prevalence of $\leq 20\%$.^{1,2} Regulatory T (Treg) cells play a key role in establishing and maintaining the balance between different branches of the immune system.^{3–5} There are currently two known populations of Treg cells. Natural or thymic

Treg cells are identified by CD4⁺CD25⁺FOXP3⁺ expression of cell surface markers. Adaptive or peripherally induced CD4⁺ type 1 regulatory T cells (T_R1) represent the second subset of Treg cells, and are defined by the expression of interleukin (IL)-10 and other cell markers (e.g. FOXP3 may be expressed only transiently).^{6,7} At the time of this investigation, the T_R1 were not yet defined in veterinary allergology. There is substantial evidence that natural Treg cells are most effective at suppressing autoreactive T-cell responses locally in noninflammatory settings, mostly by direct cell-to-cell contact. By contrast, during self-damaging inflammatory reactions to different external antigens that are more similar to the infectious setting, adaptive Treg cells might be induced to suppress the pathological immune responses, predominantly by producing immunosuppressive cytokines such as transforming growth factor (TGF)- β and IL-10.⁸ Nevertheless,

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distinguishing between Treg cell subsets (especially natural versus adaptive) is challenging.⁹ Measuring the amount of demethylation of the *FoxP3* gene may provide insight in the stability of FoxP3 expression and thereby distinguish natural Tregs from adaptive Treg cells and activated conventional T cells.^{8,10} Recently it was shown that Helios, a transcription marker, and a good marker of stable and highly suppressive Treg cells in mice and people, although there is still controversy regarding its utilization as a reliable marker in distinguishing natural and adaptive Treg cells.^{11,12} Data on the use of Helios as a Treg marker in dogs are very limited.

In the peripheral blood of human allergic patients, the numbers and function of different Treg cell phenotypes have variously been reported to be equivalent, higher or reduced, relative to nonatopic controls.^{10,13–17} By contrast, adult allergic dogs exclusively showed elevated Treg cell levels in the peripheral blood.^{18–20} Elevated Tregs also were demonstrated in an experimental model of canine AD.²¹

Current knowledge about Treg cells early in life and their possible role in allergy initiation is limited. One study showed that children who developed allergic conditions had reduced numbers of CD4 + CD25+Foxp3 + cells, reduced Foxp3 expression and a lower IL-10 production after antigen stimulation of their cord blood samples compared to children without allergic conditions.²² Genetic mutations of the forkhead box protein (Foxp3) modify Treg development and may have serious consequences, as demonstrated by IPEX (Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked) syndrome.²³ Additional genetic variation in Treg cell activity independent of the IPEX mutation has been identified, a relevant example being a reduction in the number of Treg cells in cord blood of infants with a hereditary risk of allergy.^{24,25} In addition to a genetic predisposition to allergic conditions, increasing evidence in people suggest that early-life exposure to gut microbiota,²⁶ microbial infections,²⁷ infestations with helminths,²⁸ contact with farm environment,²⁹ farm milk consumption⁵ and serum vitamin D³⁰ levels may influence the development of the T-regulatory immune system. To date, Treg cell biology in early stages of sensitization and allergy development has not been elucidated in veterinary medicine. Therefore, this study longitudinally quantified Treg cell subsets from birth up to adult age and explored a possible association with allergic sensitization and AD development in a birth cohort of West Highland white terriers (WHWT). To better elucidate different Treg responses in allergic versus healthy dogs, the contribution of several environmental factors in the early life period (3 months of age) was assessed.

Methods and materials

Study population and collection of blood samples

We prospectively studied a subgroup of dogs participating in a birth cohort, aiming at investigating the natural history of AD and the influencing early-life factors, as described in detail elsewhere.^{31,32} Briefly, within 18 months from June 2014, 107 WHWT puppies from 29 litters and 17 breeders were recruited. All breeders, and subsequently the new owners, signed a written informed consent before participating in the study, which was approved by the the Committee on Animal Experimentation [Kantonale Tierversuchskommission

(r-KTVK)] of the Canton of Zurich Department of Health ZH199/201/4, no. 25693 and conducted in accordance with guidelines established in Swiss legislation (Federal Animal Protection Law 455).

All puppies were followed prospectively to assess whether they developed AD, starting with a clinical examination at six to eight weeks of age and continuing until the age of three years. The disease severity was determined at the age of 3 years, as published previously.³¹ Peripheral blood samples were obtained at 3 months of age (n = 71), and in some cases, with consent of the new owners, also at 3–12 months (n = 26) and 12–36 months (n = 16) of age.

Data collection

Blood sample preparation and flow cytometry

Blood was collected into EDTA and serum coagulation tubes. Peripheral blood mononuclear cells (PBMCs) were prepared and stored as published previously.³³ Serum was stored at –80°C for further serological analyses.

Cryopreserved PBMC samples were batch-analysed using previously published and validated techniques.³³ All analytical antibodies were obtained from eBioscience (Vienna, Austria). The following antibodies with corresponding isotype controls were used: CD4, CD25, Foxp3, IL-10 and Helios. (See Table S1 for more details on antibodies.) The Foxp3 staining buffer set (eBioscience) was used for intracellular staining of the nuclear factors Foxp3 and Helios, and the intracytoplasmic cytokine IL-10. Dead cells were routinely excluded from the analyses, which were restricted to single cells using FSC-H and FSC-A signals. Three populations of Treg cells were gated and calculated: CD4⁺CD25⁺Foxp3⁺, CD4⁺CD25⁺Foxp3⁺Helios⁺ and CD4⁺IL-10⁺. Stained cells were analysed on a Gallios 10/3 flow cytometer (Beckman Coulter; Nyon, Switzerland) and data were analysed using FlowJo acquisition software (Tree Star Inc.; Ashland, OR, USA).

Determination of total, house dust mite (HDM)- and *Toxocara canis*-specific immunoglobulin (Ig)E and *T. canis*-specific IgG at 3 months of age

Total serum IgE concentrations in ng/mL were evaluated using a commercial dog IgE ELISA quantitation kit (Bethyl Laboratories; Montgomery, AL, USA), as described previously.²¹ Additionally, *Der-matophagoides farinae* (Der f), *D. pteronyssinus* (Der p) and Der f 2-specific IgE concentrations in the serum (in ng/mL) were quantified using an allergen-specific IgE ELISA (Animal Life Sciences Laboratories, ZENOAG; Tokyo, Japan). IgG and IgE antibodies against larval *T. canis* excretory and secretory (E/S) antigen were evaluated using an ELISA method described previously.²¹

Skin microbiome

Skin microbiota samples of 3-month-old puppies were obtained and processed as described previously.³² The samples were subjected to 16S rRNA gene amplicon sequencing to disclose the bacterial skin microbiota and bioinformatic analysis was performed as described previously.³²

Indoor allergen and endotoxin sampling, extraction and determination

Dust was collected from the environment of 3-month-old puppies and the endotoxin and mite proteins were extracted sequentially in a standardized manner by the investigators as reported previously.^{31,34,35}

Endotoxin was assayed in duplicate according to the manufacturer's instructions with a quantitative kinetic limulus amoebocyte lysate (LAL) method, using the same batch of LAL reagents and standards for all analyses (BioWhittaker; Walkersville, MD, USA). The concentration was calculated as the mean of the two values and was expressed in IU/mL and then calculated to IU of endotoxin/bedding sample. Der p 1 and Der f 1 were measured by a two-site monoclonal antibody (mAb) ELISA (Indoor Biotechnologies; Cardiff, Wales, UK) using the anti-Der p1 monoclonal 5H8 (clone 5H8 C12 D8) and anti-

Der f1 6A8 (clone 6A8 B10 D12) antibodies, as described previously.³⁶ Measured levels of HDM allergens were calculated as µg of allergen/bedding sample.

Statistical analysis

Normality in distribution of data was tested using the Kolmogorov–Smirnov test. The Wilcoxon matched-pairs signed rank test (nonparametric data) was used to compare the data of the allergic and nonallergic dogs at each age time point (3 months, 3–12 months and 1–3 years); within-groups changes over time were calculated in the same manner. The relationship between the Treg proportions and different influencing factors was assessed. For continuous variables (dust amount in the bedding, endotoxin and HDM protein levels in bedding dust, HDM-specific IgE, total IgE, *T. canis*-specific IgE levels, disease severity, maternal Treg cells, the relative abundance of the three most abundant genera of the puppies' skin) a Spearman's rank correlation coefficient *r* was used. For noncontinuous variables (urban versus rural environment, *T. canis*-specific IgG positive serology, early disease onset) Fisher's exact tests were performed. Statistical analysis was performed using Prism v7 software (GraphPad; La Jolla, CA, USA); *P*-values ≤ 0.05 were considered statistically significant.

Results

Viability of PBMC samples

After thawing, only 38 of 71 samples collected at 3 months of age had sufficient cell numbers to perform flow cytometric analysis for the different Treg cell subsets (recovery rate 54%). Between 3 months and 1 year of age, PBMC samples from 26 dogs were obtained, with a similar recovery rate as above. In dogs >1 year old, samples from only 16 dogs were obtained, with a recovery rate of 100%.

Longitudinal analysis of peripheral blood levels of CD4⁺CD25⁺Foxp3⁺ Treg cell populations in healthy and atopic dogs

The percentages of peripheral blood CD4⁺CD25⁺Foxp3⁺ cells of healthy dogs were significantly higher at age 3 months (*P* = 0.021) and ≤1 year (*P* = 0.028) compared to PBMCs of allergic dogs (Figure 1). There was a mean of 4.5% in healthy dogs versus 3.3% in allergic dogs at age 3 months, and 4.0% in healthy versus 2.9% in allergic dogs aged 3–12 months. In dogs >1 year old, the percentages of Treg cells were reversed (mean 1.6% in healthy dogs versus 3.7% in allergic dogs). This difference in the cell population approached significance (*P* = 0.053). The percentage of Treg cells did not change significantly over time in allergic dogs. However, in healthy dogs, there was a statistically significant decrease of 64% in CD4⁺CD25⁺Foxp3⁺ cells (mean 4.5 to 1.6%) when comparing values of 3-month-old healthy puppies and 1–3-year-old healthy adult animals (*P* = 0.003).

Longitudinal analysis of functional peripheral blood Treg cell markers, IL-10 and Helios

There was no difference in peripheral blood CD4⁺IL-10⁺ and Helios⁺CD4⁺CD25⁺Foxp3⁺ cell percentages between allergic and healthy dogs at any of the observed time points (Figure 2a,b). In addition, within both the allergic and healthy groups there was a significant reduction of Helios⁺CD4⁺CD25⁺Foxp3⁺ cell proportions over time.

Mean reduction compared to starting levels was 10.2% (*P* = 0.013) in healthy and 6.2% (*P* = 0.024) in allergic dogs.

Relationship between environmental exposure during early life and peripheral blood Treg cell proportions

The values for the possible influencing factors are shown in Table S1.

Peripheral blood CD4⁺CD25⁺Foxp3⁺ cells were associated neither with endotoxin or HDM allergen levels in bedding, nor with HDM-specific, *T. canis*-specific and total IgE blood levels measured during the first three months of life. The Spearman's correlations did not reveal a significant association between Treg cells and *T. canis*-specific IgE serum levels (*r* = −0.147, *P* = 0.616 in healthy dogs; *r* = 0.21, *P* = 0.373 in allergic dogs). The influence of *T. canis* infections was not calculated, as only two dogs per group had *T. canis*-specific IgG levels above the cut-off level of the test, which was determined in a previous study.²¹ In our previous study in the same birth cohort, we determined *Streptococcus*, Lachnospiraceae family unclassified genus and *Acinetobacter* as the most abundant bacterial genera of the early-life skin microbiome.³² The Spearman's rank correlation coefficient indicated that there was a moderate, yet significantly positive association between the relative abundance of Lachnospiraceae and the percentage of CD4⁺CD25⁺Foxp3⁺ cells in puppies that became allergic (*r* = 0.568, *P* = 0.017) (Figure 3), and not in nonallergic puppies (Table 1).

Discussion

To the best of our knowledge, this is the first study to examine the relationship between early-life peripheral blood Treg cells and spontaneous development of AD. In this birth cohort of WHWT dogs, we demonstrated that the blood Treg cells in early life were significantly lower in dogs which later developed allergic disease, compared to dogs which remained healthy. Similar results were shown in a study comparing Treg levels in children from a low-risk (Estonian) and high-risk (Finnish) population, with the latter population having significantly lower neonatal blood Treg cell levels.²⁶ This contrasts with other studies, one showing that children from allergic mothers have higher Treg cell levels in the cord blood,³ and others not finding any significant differences.^{37,38} Our results are consistent with those from studies of people, suggesting that an increase in Treg cells in early life is important for adaptation to environmental change after birth and very likely also for disease prevention.³⁹

When the clinical diagnosis of AD was established in this study, Treg cell levels were significantly higher in the allergic group in comparison to cell populations in the healthy group. This is in line with previous studies in people³⁷ and dogs,^{18–20} suggesting a possible secondary compensatory mechanism to counteract the inflammatory response, as was shown in our previous experimental canine AD study.²¹ Interestingly, the Treg cell levels of allergic dogs never reached nor exceeded the early life (3–12 months of age) Treg cell levels of the healthy dogs.

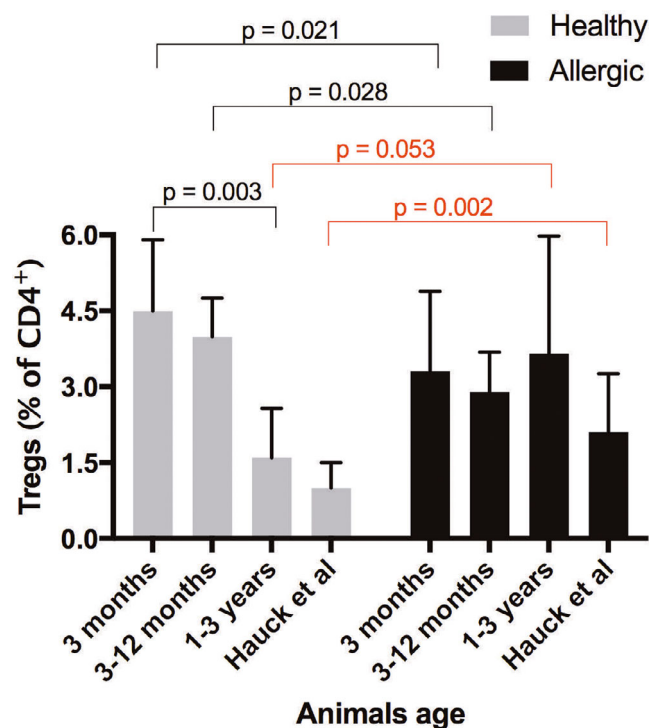


Figure 1. Longitudinal follow-up of peripheral blood CD4⁺CD25⁺Foxp3⁺ regulatory T (Treg) cells in healthy and allergic dogs.

At 3 months of age, healthy dogs had statistically significant higher levels of peripheral blood CD4⁺CD25⁺Foxp3⁺ cells compared to the allergic dogs. This relationship remained significantly different up to 3 years of age and reversed after 1 year of age (indicated in red) when the atopic dogs showed significantly more CD4⁺CD25⁺Foxp3⁺ than the healthy dogs. Additionally, there was a significant reduction in this cell population within the healthy group, which was not found in the atopic group. The results at 1–3 years old are in line with our previous study¹⁸ in which adult dogs (mean age 4.3 years) with spontaneous atopic dermatitis (AD) were analysed, as shown by the fourth column in this figure.

Additionally, we showed changes in the Treg cell population during the maturation of animals, with a significant reduction in the healthy group from adolescence to adulthood. Similar longitudinal studies in people, showed a significant increase in Treg cells at three to five days after birth, with a steady decline up to 3 years of age.⁴⁰ Overall, the Treg cell values of both allergic and healthy dogs in this study were within the expected range reported for people and dogs previously.^{18,41}

In our study, the Treg cell populations utilizing either the IL-10 or the Helios functional marker were not significantly different between allergic and healthy groups. This suggests that the functional capacity of these cells was not compromised and indicates a very stable and functional cell population. Additionally, the Helios analysis showed that >90% of the CD4⁺CD25⁺Foxp3⁺ Treg cell population were Helios-positive. This was true for both healthy and allergic dogs at all time points. The levels of Helios⁺ Treg cells in our study were very similar to those reported in mice and people.¹¹ A previous study in healthy dogs showed that only 50% of Treg cells expressed the Helios marker,⁴² very likely as a consequence of different study methodology. In addition, the mild yet significant longitudinal reduction in the Helios⁺ Treg cell population in our study dogs could be explained by the enhanced generation of peripherally induced Treg cells in the adult dogs.

Several epidemiological studies have demonstrated a critical role for environmental factors in the development of allergic diseases. For example, rural environments

were shown to be a natural model of allergy protection in people⁴³ and increased Treg cell levels were inversely associated with the occurrence of allergic asthma in children.^{5,44} Unfortunately, in our study the animals were not exposed to farm environments, so we were unable to test this correlation in dogs. Furthermore, inverse associations between environmental endotoxin, a marker for microbial exposure and development of allergic disease were reported in people and adult dogs.^{34,44} Herein we found no significant associations between environmental endotoxin and peripheral blood Treg cell levels in either allergic or healthy dogs. Possible explanations for this are a very similar environment of all participating dogs (similar hygiene levels, most dogs living in a rural environment),³¹ a too-small sample size or microbial agents other than endotoxins [for example fungal $\beta(1,3)$ -glucans, muramic acid] that could have influenced the observed T-regulatory responses.

In agreement with a recent publication,²⁷ the early-life skin microbiota of dogs involved in this cohort study was not associated with development of AD. In atopic people, the relative abundance of both *S. aureus* and *S. epidermidis* is reported to be elevated and the abundance of *Propionibacterium* and other genera (*Streptococcus*, *Acinetobacter*, *Corynebacterium* and *Prevotella*) is reduced.⁴⁵ Canine AD is associated with reduced bacterial diversity, and with increased abundance of *Staphylococcus pseudintermedius* and *Corynebacterium* spp. on the skin.⁴⁶ The most abundant genera of the early-life skin microbiome described in our previous study³² were not

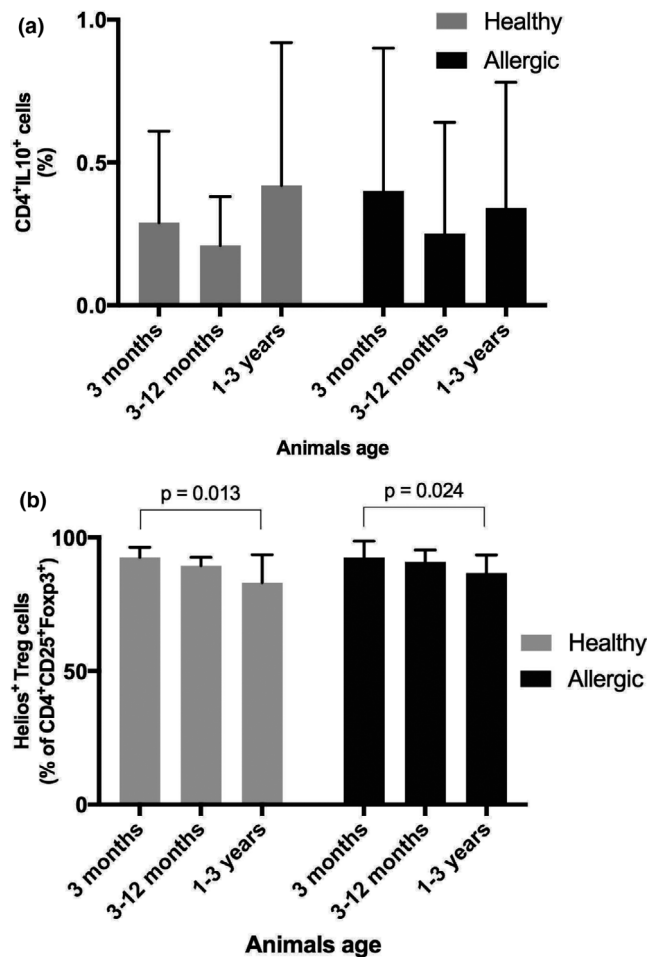


Figure 2. Longitudinal follow-up of (a) peripheral blood CD4⁺IL-10⁺ cells and (b) peripheral blood Helios⁺ regulatory T (Treg) cells in healthy versus allergic dogs.

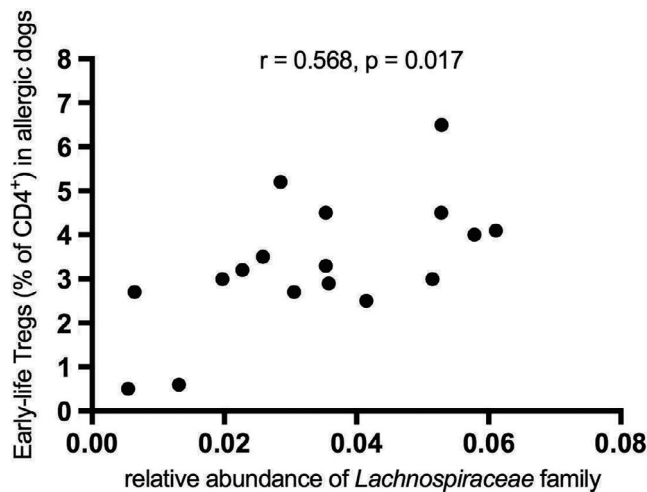


Figure 3. The correlation between the relative abundance of Lachnospiraceae in the early-life skin samples and peripheral blood regulatory T (Treg) cell levels in allergic dogs.

associated with the Treg cell levels in either healthy or allergic dogs except for a significant positive correlation between the relative abundance of Lachnospiraceae on the skin and the percentage of CD4⁺CD25⁺Foxp3⁺ Treg cells from allergic dogs. Genera belonging to Lachnospiraceae have been positively associated with egg

allergy in children.⁴⁷ This finding contrasts with another study in which the depletion of Lachnospiraceae in the gut was associated with lower relative abundances of genes associated with immune development.⁴⁸ The importance of this finding has to be determined in further studies. Additionally, the systemic regulatory effects of

Table 1. Correlation between the regulatory T cells of healthy and allergic dogs and the three most abundant genera of the early-life skin microbiome

Bacterial genus	Puppy group by health status	Spearman's rank correlation coefficient <i>r</i> (<i>P</i> -value)		
		CD4 + IL-10+(%CD4 + CD25+Foxp3 + cells)	CD4 + CD25+Foxp3 + Helios+ Tregs Treg cells (%CD4+)	Treg cells (%)
<i>Streptococcus</i>	Healthy	-0.068 (0.842)	0.209 (0.537)	-0.515 (0.105)
	Allergic	-0.044 (0.866)	0.270 (0.294)	-0.022 (0.933)
Lachnospiraceae family unclassified genus	Healthy	-0.319 (0.339)	0.182 (0.593)	-0.405 (0.216)
	Allergic	0.568 (0.017)	0.148 (0.570)	-0.140 (0.592)
<i>Acinetobacter</i>	Healthy	-0.173 (0.611)	-0.082 (0.811)	-0.046 (0.894)
	Allergic	-0.232 (0.370)	-0.140 (0.593)	-0.121 (0.643)

Bold value is statistically significant.

Acinetobacter on the skin were studied in people, showing a mild positive association with IL-10 expression in PBMCs from healthy individuals,⁴⁹ by contrast to our study in which we found a negative, albeit not significant correlation between *Acinetobacter* and Treg cells of healthy and allergic dogs.

The hygiene hypothesis suggests that not only microbial exposure, but also exposure to helminths play an important role in the development of allergic conditions in people.⁵⁰ As reviewed recently in both people and dogs, the ability of helminths to induce parasite-reactive Treg cells and IL-10 production may occur through parasite E/S product.^{28,51} Therefore, we performed IgG and IgE serology directed against the E/S antigens of *T. canis*, one of the most abundant canine endoparasites. *Toxocara canis*-specific serum IgE levels were not significantly associated with Treg cells in this study. *T. canis*-specific IgG were not calculated due to the low number of positive serological samples.

Increasingly it is recognized that the timing, load and route of allergen exposure affect allergic disease phenotypes and development.⁵² The relationship between HDM allergens and T-regulatory responses was characterized mainly through observations of responses to allergen-specific immunotherapy, which involves upregulation of activated allergen-specific Treg cells and downregulation of dysfunctional allergen-specific Treg cell subsets.⁵³ In this study we could not find any significant associations between peripheral blood Treg cell values and early-life HDM levels in the environment. Therefore, one could say that HDM are not primary drivers of the regulatory arm of immunity, a conclusion which is further supported by a previous study ruling out HDM as a major factor in the causality of atopic eczema in people.⁵⁴

The strength of this study was the use of a large cohort to prospectively investigate Treg cell populations and their relationship to various environmental exposures from early life through to immune maturation. One limitation of the present study is that only the percentage and not the absolute Treg cell numbers were determined. Further, the functionality of Treg cells was assessed only partially (using IL-10 and Helios). There are functional assays and other markers which could possibly improve estimation of the functionality of these cells. These include TGFβ, PD-1, CTLA-4, GITR and the Treg-specific demethylated region (TSDR).³ Soluble regulatory factors, such as the cytokines IL-10 and TGFβ might have been

measured. However, this was not possible owing to the relatively small volumes of blood collected from the puppies and the requirement to use these samples for other serological analyses. Additionally, it would have been advantageous to follow the time-dependent Treg cell values on each individual. Unfortunately, this was not possible as we obtained only one of the planned blood samples for the majority of the study subjects.

Despite the enrolment of a large cohort of dogs, a relatively low sample number could explain the lack of association between Treg cell levels and the different environmental factors. The main reason for this was the low recovery rate (50%) after thawing, even though an optimised protocol was used.³³ We chose to use cryopreserved material as it is particularly useful in longitudinal studies. Frozen samples allow batch testing in the same laboratory, resulting in a significant reduction of interlaboratory and interassay variability. In the future, larger sample quantities should be used, taking into account the aforementioned recovery rates. The main cause for the low number of samples at the later time points was the lack of owner consent/compliance or that the re-check at this point was not conducted in-person at the clinic.

Taken together these results suggest an important role of Treg cells in the initiation/prevention of AD. Not only sufficient Treg levels, but also their timely development, are necessary to maintain immunological homeostasis. This area of research merits more attention in the future. Of particular interest is the identification of effective methods to exploit the potential of Treg cells in relation to prevention and treatment of this prevalent disease.

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Supporting Information

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

Table S1. List of antibodies used for canine Treg cell flow cytometry.

Table S2. Data on Treg cell populations, allergenic and infectious factors and clinical 403 disease data.

Résumé

Contexte – Les cellules Treg sont impliquées dans l'homéostasie de la régulation immunitaire et la suppression de l'inflammation et de la polarisation cellulaire T. La connaissance actuelle du rôle des cellules T reg dans l'initiation de l'allergie est limitée chez le chien comme chez l'homme.

Objectifs – Explorer le rôle des cellules T reg circulantes et leur possible facteurs d'influence sur le développement de la dermatite atopique (AD).

Méthodes – Cette étude suit une part de cohorte de naissance du West Highland terrier et les classe selon d'éventuels signes cliniques d'AD (i.e. allergique versus sain). Les phénotypes Treg sont évalués longitudinalement par cytométrie de flux à l'âge de 3, 3-12 et 12-36 mois, et associés avec le développement de l'AD. Différents facteurs antigéniques de vie précoce (endotoxines et allergènes de la poussière de maison, immunoglobuline (Ig)E/IgG spécifiques de *Toxocara canis*, IgE totaux et spécifiques d'allergènes, microbiote cutané) ont été mesurés à l'âge de trois mois, et une association possible avec les niveaux de cellules Treg a été évaluée.

Résultats – Les pourcentages de cellules Treg CD4+CD25+Foxp3+ chez les chiens sains étaient significativement plus élevés chez les chiens à l'âge de 3 mois (moyenne 4.5% sain versus 3.3% allergique; $P = 0.021$) et <1 an (4.0% sain versus 2.9% allergique; $P = 0.028$) lorsque comparés aux pourcentages de cellules Treg chez les chiens qui ont développés l'AD. Il y a avait une corrélation positive significative entre la relative abondance de Lachnospiraceae sur la peau et les cellules Treg CD4+CD25+Foxp3+ des chiots devenus allergiques ($r = 0.568$, $P = 0.017$).

Conclusion et importance clinique – Des études à plus large échelle sont nécessaires pour identifier la valeur pratique de ces données dans le diagnostic, le traitement et la prévention de l'AD.

Resumen

Introducción – las células T reguladoras (Treg) participan en la homeostasis de la regulación inmunitaria y de la supresión de la inflamación, y en la polarización de las células T. El conocimiento actual sobre el papel de las células Treg en el inicio de una enfermedad alérgica es limitado tanto para las personas como para los perros.

Objetivos – Explorar el papel de las células Treg circulantes y sus posibles factores de influencia en el desarrollo de la dermatitis atópica (AD).

Métodos – este estudio siguió parte de unas camadas de perros West Highland White Terrier y los clasificó de acuerdo con los posibles signos clínicos de la AD (es decir, alérgico frente a saludable). Los fenotipos Treg se evaluaron longitudinalmente mediante citometría de flujo a los 3, 3–12 y 12–36 meses de edad, y se asociaron con el desarrollo de AD. Se midieron diferentes factores antigénicos en la vida temprana [endotoxinas y alérgenos en el polvo doméstico, inmunoglobulina (Ig)E/IgG específica de *Toxocara canis*, IgE total y específica de alérgeno, microbiota cutánea] a los tres meses de edad y se evaluó una posible asociación con niveles de células Treg.

Resultados – los porcentajes de células Treg CD4+CD25+Foxp3+ en perros sanos fueron significativamente más altos a los 3 meses de edad (media 4,5% sanos frente a 3,3% alérgicos; $P = 0,021$) y <1 año (4,0% sanos versus 2,9% perros alérgicos; $P = 0,028$) en comparación con los porcentajes de células Treg en perros que desarrollaron AD. Hubo una correlación significativamente positiva entre la abundancia relativa de Lachnospiraceae en la piel y las células Treg CD4+CD25+Foxp3+ en cachorros que desarrollaron alergia ($r = 0,568$, $P = 0,017$).

Conclusión e importancia clínica – se necesitan más estudios a gran escala para identificar el valor práctico de estos hallazgos en el diagnóstico, el tratamiento y la prevención de la AD.

Zusammenfassung

Hintergrund – Die regulatorischen T (Tregs) Zellen sind bei der Homöostase der Immunregulierung und der Supprimierung der Entzündung und Polarisierung der T-Zellen involviert. Das momentane Wissen in Bezug auf die Rolle der Tregs Zellen beim Auslösen allergischer Erkrankungen ist auf Menschen und Hunde beschränkt.

Ziele – Eine Erforschung der Rolle der zirkulierenden Tregs Zellen und ihrer möglichen Einflussfaktoren auf die Entwicklung einer atopischen Dermatitis (AD).

Methoden – Diese Studie folgte einer Geburtskohorte von West Highland White Terriern und teilte sie entsprechend ihrer klinischen Zeichen von AD (i.e. allergisch versus gesund) ein. Die Treg Phänotypen wurden longitudinal mittels Flowzytometrie nach 3, 3-12 und 12-36 Altersmonaten erfasst, und mit der Entwicklung der AD in Zusammenhang gestellt. Unterschiedliche Antigenfaktoren im frühen Leben [Endotoxine und Allergene im Hausstaub, *Toxocara canis*-spezifisches Immunglobulin (Ig)E/IgG, Allergen-spezifisches und Total IgE, Haut Mikrobiota] wurden in einem Alter von drei Monaten gemessen und ein möglicher Zusammenhang mit der Anzahl der Tregs Zellen untersucht.

Ergebnisse – Die Prozentanteile von CD4+CD25+Foxp3+ Treg Zellen waren bei gesunden Hunden signifikant höher als bei 3 Monate alten (Durchschnitt 4,5% gesunde versus 3,3% allergische; $P = 0,021$) und <1 Jahr alten (4,0% gesunde versus 2,9% allergische; $P = 0,028$) Hunden im Vergleich zum Prozentsatz an Treg Zellen bei Hunden, die AD entwickelten. Es bestand eine signifikant positive Korrelation zwischen der relativen Häufigkeit von Lachnospiraceae auf der Haut und CD4+CD25+Foxp3+ Treg Zellen bei Welpen, die Allergien entwickelten ($r = 0,568$, $P = 0,017$).

Schlussfolgerungen und klinische Bedeutung – Weitere großangelegte Studien sind nötig, um den praktischen Wert dieser Ergebnisse bei der Diagnose, der Behandlung und der Prävention von AD zu identifizieren.

要約

背景 – 制御性T(Treg)細胞は、免疫調節の恒常性の維持、炎症やT細胞の分極抑制に関与している。アレルギー疾患の発症における Treg 細胞の役割についての現在の知見は、人でも犬でも限られている。

目的 – 本研究の目的は、アトピー性皮膚炎 (AD) の発症における循環Treg細胞の役割およびその影響因子の可能性を探る事であった。

方法 – 本研究では、ウエスト・ハイランド・ホワイト・テリアの出生コホートの一部を追跡調査し、最終的なADの臨床症状 (アレルギー対健常) に応じて分類した。Tregの表現型を、生後3ヶ月、3~12ヶ月、12~36ヶ月のフローサイトメトリー法で縦断的に評価し、AD発症と関連付けた。異なる初期の抗原性因子 (ハウスダスト中のエンドトキシンやアレルゲン、*Toxocara canis*特異的免疫グロブリン (Ig)E/IgG、アレルゲン特異的IgEおよび総IgE、皮膚微生物叢) を生後3ヵ月で測定し、Treg細胞レベルとの関連性の可能性を評価した。

結果 – 健常犬のCD4+CD25+Foxp3+ Treg細胞の割合は、AD発症犬のTreg細胞の割合と比較して、生後3ヶ月 (平均4.5%、アレルギー3.3%、 $P=0.021$) および1歳未満 (4.0%、アレルギー2.9%、 $P=0.028$) の犬で有意に高かった。アレルギー発症の子犬の皮膚上のラクノスピラ科の相対量およびCD4+CD25+Foxp3+ Treg細胞間には有意な正の相関があった ($r = 0.568$, $P = 0.017$)。

結論と臨床上の重要性 – ADの診断、治療、予防におけるこれらの知見の実用的価値を明らかにするためには、さらなる大規模な研究が必要である。

背景 – 調節性T(Treg)細胞参与免疫調節の稳态以及炎症和T细胞极化的抑制。目前关于Treg细胞在过敏性疾病初期的作用,人和犬的相关知识都有限。

目的 – 探索循环Treg细胞及其可能的影响因素在特应性皮炎(AD)发生中的作用。

方法 – 本研究跟踪了一批相同血统的西高地白梗犬,并根据AD的最终临床体征对其进行分类 (即过敏与健康)。在3、3-12和12-36月龄时,通过流式细胞术纵向评估Treg表型,以及发生AD的相关性。在3月龄时检测不同的生命早期抗原因子[屋尘的内毒素和过敏原、弓首蛔虫特异性免疫球蛋白(Ig)E/IgG、过敏原特异性和总IgE、皮肤微生物群],并评估与Treg细胞水平的可能关联。

结果 – 与AD患犬的Treg细胞百分比相比,3月龄 (健康犬平均4.5%与过敏犬3.3%; $P=0.021$) 和 <1岁 (健康犬4.0%与过敏犬2.9%; $P=0.028$) 健康犬的CD4+CD25+Foxp3+Treg细胞百分比显著更高。过敏幼犬皮肤上,毛螺旋菌相对丰度与CD4+CD25+Foxp3+Treg细胞呈显著正相关($r=0.568$, $P=0.017$)。

结论和临床重要性 – 需要更多大规模研究来确定这些发现在AD诊断、治疗和预防中的实用价值。

Resumo

Contexto – As células T reguladoras (Treg) estão envolvidas na homeostase da regulação imunológica e na supressão da inflamação e polarização das células T. O conhecimento atual sobre o papel das células Treg no início da doença alérgica é limitado tanto para pessoas quanto para cães.

Objetivos – Explorar o papel das células Treg circulantes e seus possíveis fatores influenciadores no desenvolvimento da dermatite atópica (DA).

Métodos – Este estudo acompanhou parte de uma coorte de nascimento de cães West Highland white terrier e os classificou de acordo com eventuais sinais clínicos de DA (alérgicos versus saudáveis). Os fenótipos Treg foram avaliados longitudinalmente por citometria de fluxo aos 3, 3–12 e 12–36 meses de idade e associados ao desenvolvimento da DA. Diferentes fatores antigênicos do início da vida [endotoxinas e alérgenos na poeira doméstica, imunoglobulina (Ig) E / IgG específica para *Toxocara canis*, IgE total e IgE alérgeno-específica, microbiota da pele] foram medidos aos três meses de idade e uma possível associação com células Treg níveis foram avaliados.

Resultados – As porcentagens de células Tregs CD4+CD25+Foxp3+ em cães saudáveis foram significativamente maiores aos 3 meses de idade (média de 4,5% saudáveis versus 3,3% alérgicas; $P = 0,021$) e em cães <1 ano (4,0% saudáveis versus 2,9% alérgicos; $P = 0,028$) quando comparados às porcentagens de células Treg em cães que desenvolveram DA. Houve uma correlação significativamente positiva entre a abundância relativa de Lachnospiraceae na pele e células Tregs CD4+CD25+Foxp3+ em filhotes que se tornaram alérgicos ($r = 0,568$, $P = 0,017$).

Conclusão e importância clínica – Mais estudos em larga escala são necessários para identificar o valor prático desses achados no diagnóstico, tratamento e prevenção da DA.

Flow cytometer channel	Antibody type	Clone	Isotype control	Clone
FL1	Anti-mouse/human Helios FITC	22F6	Arm hamster IgG FITC	eBio299Arm
FL2	Anti-canine CD25 PE	P4A10	Mouse IgG1K PE	eB149/10H5
FL5	Anti-canine CD4 PE-Cyanine7	YKIX302.9	Rat IgG2b K PE-Cyanine7	P3.6.2.8.1
FL6	Anti-mouse/rat Foxp3 eFluor 660	FJK-16s	Rat IgG2a K eFluor 660	eBR2a
FL8	Fixable Viability Dye eFluor 780	JES-9D7		
FL9	Anti-human IL10 PerCP-eFlour®710	JES3-9D7	Rat IgG1 eFlour®710	eBRG1