

Antibody levels to *Malassezia pachydermatis* and *Staphylococcus pseudintermedius* in atopic dogs and their relationship with lesion scores

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Background – Elevated immunoglobulin E (IgE) levels to *Malassezia* or *Staphylococcus* species in human atopic dermatitis are related to the skin severity index; a similar association has not been reported in atopic dogs.

Objectives – To investigate serum levels of allergen-specific IgE, total specific IgG and IgG subclasses (IgG1 and IgG2) for *M. pachydermatis* and *S. pseudintermedius*, and to correlate them with the severity of dermatitis in dogs.

Animals – Serum samples were collected from dogs categorized by age and disease status. Groups 1 and 2: <3-year-old healthy (n = 9) and atopic dogs (n = 9), respectively; and groups 3 and 4: ≥3-year-old healthy (n = 11) and atopic dogs (n = 14), respectively.

Methods and materials – Antibody levels were measured by ELISA. The Canine Atopic Dermatitis Lesion Index (CADLI) was analyzed in relation to antibody levels.

Results – Specific IgE and total IgG against *M. pachydermatis* and *S. pseudintermedius* were significantly increased in atopic dogs of all ages. Although differences between atopic and healthy dogs, with regard to specific IgG1 and IgG2 levels to each microbe, varied in significance within age groups. No significant relationships were found between the CADLI and any specific immunoglobulin levels for both microbe types.

Conclusions and clinical importance – In dog skin, microbes may act as allergens triggering inflammatory responses via IgE- and IgG-dependent pathway(s). The affinity of the IgG subclass produced may vary according to antigen type. Specific IgE levels may be related to clinical disease in dogs and not to skin lesion severity.

Introduction

Canine atopic dermatitis (cAD) is an inflammatory skin disease that is associated with allergen-related immunoglobulin E (IgE) antibodies.¹ Concurrent carriage of both *Malassezia pachydermatis* and *Staphylococcus pseudintermedius* in atopic dogs has been related to specific IgE production.^{2,3} In human AD, specific IgE levels to *Malassezia* species or *Staphylococcus* species are reported to be related to the skin severity index;^{4,5} such an association has not been reported for cAD.

Four IgG subclasses have been described in humans, and each subclass is associated with different types of

antigens and has different effector functions.⁶ In atopic humans, IgG1 and IgG4 levels are related to allergen sensitization with high specific IgE levels, simultaneously,^{7,8} whereas the IgG2 response is restricted to polysaccharide antigens.⁸ In dogs, four subclasses of IgG have been reported. One study reported elevated IgG2 levels with decreasing IgG1 levels in sera from dogs with a range of immune-mediated or inflammatory diseases.⁹ Another study reported on allergen-specific IgG subclass and suggested that the response depended on the nature of the allergen, and that *Demodex farinae*-IgG1 levels were rarely detected in atopic dogs.⁹ A further study reported a high level of IgG1 against *D. farinae* in atopic dogs which increased after immunotherapy.¹⁰ Due to a lack of availability of commercial anti-dog IgG subclass antibodies, only IgG1 and IgG2 have been studied.^{11,12}

The aim of this study was to investigate levels of allergen-specific IgE, IgG1 and IgG2 directed against *M. pachydermatis* and *S. pseudintermedius*, with total IgG levels, then correlate them with lesion severity in

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cAD as defined by the Canine Atopic Dermatitis Lesion Index (CADLI).¹³

Methods and materials

Study population

The animal-use protocol was approved under the Institutional Animal Care and Use Committee of Chulalongkorn University Animal Care and Use Protocol (CU-ACUP); Animal Use Protocol no. 1731046. Four groups of dogs were enrolled; healthy dogs were categorized into groups 1 (<3-year-old; $n = 9$) and 3 (≥ 3 -year-old; $n = 11$), respectively. Atopic dogs were categorized into groups 2 (<3-year-old; $n = 9$) and 4 (≥ 3 -year-old; $n = 14$), respectively (Table S1). Atopic dogs were defined according to published guidelines.¹ All atopic dogs had suffered from recurrent infections with *M. pachydermatis* and/or *S. pseudintermedius*, based on cytological findings.^{14–16} Cutaneous adverse food reaction was ruled out by elimination diet for a minimum of eight weeks. After successful treatment with antimicrobial therapy and two weeks withdrawal of anti-inflammatory, antipruritic and antimicrobial drugs (none of the dogs had been treated with steroids),¹⁷ intradermal testing (IDT) with environmental allergens was performed according to previous reports.¹⁸ All atopic dogs had at least one positive intradermal reaction to an allergen. The severity of the skin lesions was determined based on the CADLI.¹³ The same investigator evaluated all subjects.

Preparation of microbe extracts

Malassezia pachydermatis strain 4180 and *S. pseudintermedius* strain AK30 were used as representative strains.^{19,20} AK30 lacked the *spa* gene so protein A could not be produced and interfere with the IgG binding assessments. The identities of both microbes were confirmed based on morphological and physiological characteristics according to routine laboratory methods.^{19,20} Colonies of *M. pachydermatis* were subcultured onto Sabouraud's dextrose agar and incubated at 35°C to obtain sufficient organisms representing all stages of the growth phase.²¹ In parallel, colonies of *S. pseudintermedius* were subcultured onto trypticase soy agar containing 5% sheep blood and incubated at 37°C. The colonies of *M. pachydermatis* and *S. pseudintermedius* were harvested individually at 72 and 24 h post-incubation, respectively, and were suspended in phosphate buffered saline (PBS; pH 7.4) at 4°C. After washing three times with PBS, the pellets were re-suspended with an equal volume of PBS, followed by adding an equal volume of SiLibeads Type S (SiLibeads; Warmensteinach, Germany). For extraction, the microbes were subjected to 10 cycles of vigorous shaking for 1 min using a Vortex Genie® 2 with replacement tube inserts (Scientific Industries; New York, NY, USA) and kept on ice for two minutes per cycles. After elution and centrifugation at 6000g for 5 min, the supernatant was filtered with Minisart filters of pore size 0.45 µm (Sartorius Stedim Biotech GmbH; Goettingen, Germany) and the protein concentration measured using a Protein Assay Kit II (Bio-Rad; Irvine, CA, USA). The filtrate was kept in aliquots at –20°C, until used.

Specific IgE ELISA

Pooled serum samples from five atopic dogs and a pool from five young healthy dogs were used in checkerboard titrations. Costar 3590 EIA plates (Corning; New York, NY, USA) were coated with microbial extracts diluted in 0.2 M BupH™ Carbonate-Bicarbonate Buffer Packs, pH 9.4 (Thermo Fisher Scientific; Rockford, IL, USA) with 2 µg of total protein used per well. After washing with PBS containing 0.05% tween 20 (PBST) plates were blocked with blocking buffer (PBST containing 1% bovine serum albumin BSA). All serum samples, antisera and conjugate were diluted in blocking buffer before being added into the wells. First, diluted serum samples (1:5) were added into wells. Next, diluted monoclonal mouse anti-dog IgE (1:2,000; clone E6-2A1, AbD Serotec; Kidlington, Oxford, England) was added, followed by diluted phosphatase conjugate (1:2,000; goat anti-mouse IgG & Human ads-AP, Southern Biotech; Birmingham, AL, USA). Finally, Sensitest Canine IgE substrate (Avacta Animal

Health; Wetherby, UK) was used for colour development. The optical density (OD) at 450 nm was measured using an ELx808 ultra reader (Bio-Tek instruments Inc., Winooski, VT, USA). After subtracting the OD of the blank control, the OD values were quantified in enzyme-linked immunosorbent assay (ELISA) units. The pooled serum samples from atopic dogs were used for calibration and were arbitrarily set at 100 units (U) as described previously.²² All samples were run in duplicate.

Total specific IgG and IgG subclasses ELISA

The IgG and IgG subclass assays were developed as described for the IgE ELISA, with some modifications. The optimal serum dilution for IgG and IgG subclass assays was 1:2,000. Diluted polyclonal sheep anti-dog IgG conjugated-Horseradish Peroxidase (HRP) (AAI32P, AbD serotec Ltd); polyclonal goat anti-dog IgG1-HRP (AHP947P, AbD serotec Ltd) and polyclonal sheep anti-dog IgG2-HRP (AHP948P, AbD serotec Ltd) in blocking buffer (1:4,000) were applied to detect allergen-specific total IgG, IgG1 and IgG2, respectively. In regard to the commercial anti-dog IgG1 and anti-dog IgG2 used for antibody detection, the manufacturer claimed there was no cross-reactivity to other IgG subclasses and these antibodies were selected for use in determining the bias of IgG subclasses in previous studies.^{11,12} Peroxidase substrate (ABTS® peroxidase substrate, KPL Inc.; Gaithersburg, MD, USA) was added for colour development. The OD of the blank control was subtracted from the OD and quantified in ELISA units.

Data and statistical analyses

GraphPad PRISM v6 GraphPad Software Incorporated; San Diego, CA, USA) was used for analysis of results. Age distributions between healthy and atopic dogs in the same category were compared by Wilcoxon–Mann–Whitney U-test. Median levels of antibody were compared by Kruskal–Wallis ANOVA Ranks with a Dunn's post hoc test. Pearson correlation (r) was used to assess relationships between CADLI scores and antibody levels in cAD subjects.

Results

Characteristics of dog samples

There were various breeds of dogs in each group (Table S1). The median ages of dogs in groups 1 and 2 were 1.67 and 1.58 years, respectively, and in groups 3 and 4, 3.85 and 4.71 years, respectively. A significant difference in age between the younger and older groups was shown ($P < 0.05$; Wilcoxon–Mann–Whitney U-test).

Reproducibility

For all *M. pachydermatis* and *S. pseudintermedius* ELISAs, the mean intra- and interassay variations did not exceed 4.88% and 10.15%, respectively.

Antibody levels to skin microbial extracts

Immunoglobulin levels for the yeast and bacteria extracts are reported in Figure 1. Atopic dogs (groups 2 and 4) showed significantly higher antibody levels to microbial extracts compared with healthy dogs (groups 1 and 3). Apart from IgG1 levels to *M. pachydermatis* in older dogs and IgG2 levels to *S. pseudintermedius* in younger dogs, all differences between healthy and atopic dogs were significant within the same age category ($P < 0.05$, ANOVA; Figure 1). There were no statistical differences within either the healthy or atopic dogs according to age (i.e. Group 1 versus 3 and Group 2 versus 4; Figure 1).

CADLI scores

The median of CADLI scores in young atopic dogs was 9 (range 0–40) whereas the median scores in aged atopic

dogs were 18 (range 4–30) (see Figure 2). There was no significant difference for CADLI scores between the atopic age groups. Two young dogs were classified as atopic and had CADLI scores of “0” on the day of sampling, when the only residual clinical sign was pruritus. The CADLI scores in atopic dogs were not correlated to any of the types of specific antibodies for either *M. pachydermatis* ($r = -0.053$ to 0.283 , $P > 0.05$) or *S. pseudintermedius* ($r = -0.385$ to -0.035 , $P > 0.05$). Overall, the IgE level to *S. pseudintermedius* had the most negative correlation to CADLI scores ($r = -0.35$, Figure 2E) even though no significant difference was found.

Discussion

This study found that both *M. pachydermatis* and *S. pseudintermedius* are capable of eliciting humoral immune responses (IgE, IgG, IgG1 and IgG2) in atopic dogs. It is possible that this humoral response is associated with the pathogenesis of cAD.^{2,3} The age of the dog was included as a factor for evaluation of antibody levels because a previous study showed that allergen-specific IgE levels increased with age in dogs with cAD.²³ However, in the present study age was not associated with a difference in specific IgE or IgG antibody levels to either micro-organism.

Overall, the increased antibody levels in atopic dogs may be a consequence of dermatitis in atopic dogs. However, the lack of standard recommendations for preparing skin microbial extracts is a limitation to confirming hypersensitivity reactions that arise from these microbes. Following a previous study,²¹ microbial colonies were harvested from subcultured plates for preparing microbial extracts. It was assumed that this would include all proteins from any growth phases, even though further quantitative immunoelectrophoretic techniques are required to find standardized allergens.

In humans and in a murine model, the IgG subclass response depends both on the antigen components and on immune deviation leading to pathogen clearance through T-cell responses.^{24–27} *Malassezia pachydermatis* organisms contain large polysaccharides and these may drive a strong IgG2 response.²⁴ This is consistent with the results herein for anti-*M. pachydermatis* IgG2, which was significantly higher in atopic dogs than in healthy controls for both age groups. However, it was found that anti-*M. pachydermatis* IgG1 was not increased in older atopic dogs compared to healthy controls. This is potentially consistent with the report that human patients with pityriasis versicolor or seborrhoeic dermatitis have an IgG1 response to *M. furfur* serovar A that is similar to healthy controls.²⁴ Differences in IgG subclass responses to *Malassezia* spp. probably are dependent on their serovars and *Malassezia*-associated dermatoses.²⁴

In the case of *Staphylococcus* species, whole cells or their components, including peptidoglycan and teichoic acid, elicit both IgG1 and IgG2 responses.^{25,26} In human patients with acne vulgaris, specific IgG1 and IgG2 to *S. epidermidis* were higher than in controls.²⁷ This is in concordance with the findings herein for IgG subclasses specific to *S. pseudintermedius*. So, the types of antigens involved may influence IgG subclass production in dogs, as suggested previously.¹¹

In vaccination models or when classifying the severity of disease, upregulation of IgG1 or IgG2 often is influenced by Th2 or Th1 responses, respectively.^{11,12,28} However, IgG subclass responses in relation to T-helper cell regulation and the role of each IgG subclass remain ambiguous in dogs. In a study of canine IgG subclass function, synthetic IgG subclass A, which bound to commercial anti-dog IgG1 antibodies, was found to have similar functions to human IgG2. While a synthetic canine IgG B subclass construct, specific for commercial anti-dog IgG2, had similar functions to human IgG1.²⁹ These findings lead to still unanswered questions about the

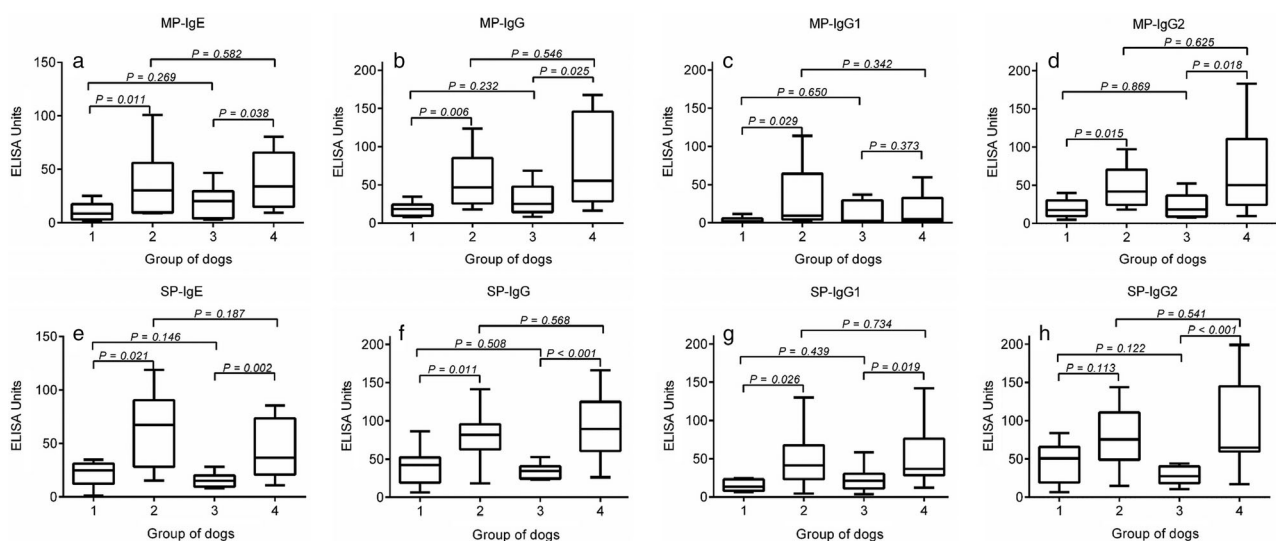


Figure 1. Median levels of specific antibody to *Malassezia pachydermatis* (MP, a-d) and *Staphylococcus pseudintermedius* (SP, e-h) in each group of dogs.

Group 1, healthy dogs (<3-year-old); Group 2, atopic dogs (<3-year-old); Group 3, healthy dogs (≥3-year-old); and Group 4, atopic dog (≥3-year-old). The median levels were compared by Kruskal–Wallis ANOVA Ranks, with significance accepted if $P < 0.05$.

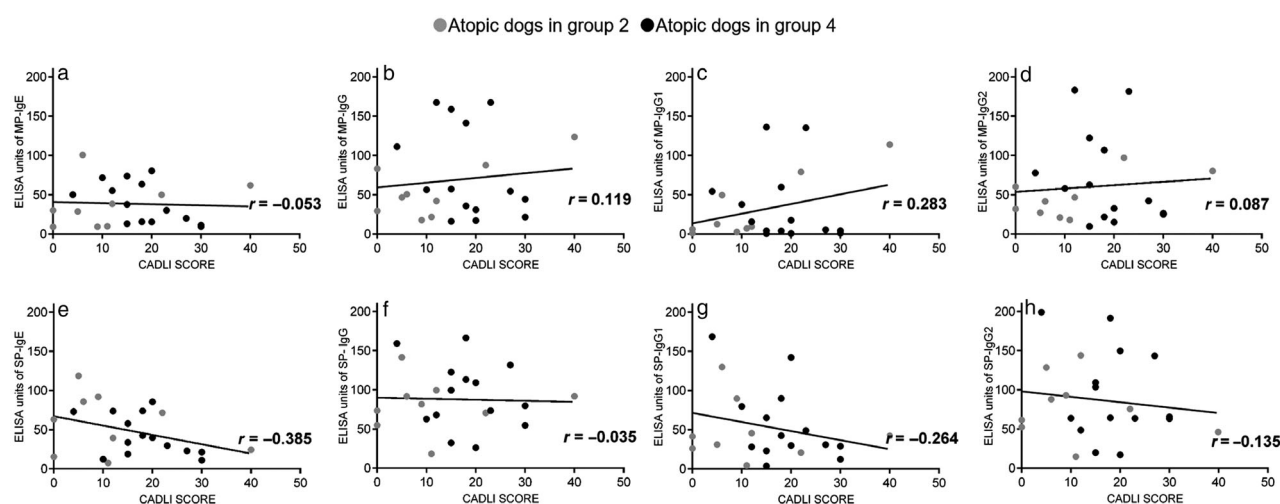


Figure 2. The Pearson correlation (r) of Canine Atopic Dermatitis Lesion Index (CADLI) scores and antibody levels to *Malassezia pachydermatis* (MP, a-d) and *Staphylococcus pseudintermedius* (SP, e-h) in atopic dogs (groups 2 and 4).

Significant differences: * with $P < 0.05$.

specificity of the commercial antibodies used in canine IgG subclass detection and differences in translation of IgG subclass responses between humans and dog. The claimed specificities of the commercial antibodies used in the present study were based on the results of previous studies.^{11,12}

T-cell regulation of immunoglobulin class expression is known to differ between species of mammals. In a murine model, IgG1 production dominated following Th2 regulation,⁷ whereas in humans the IgG1 response was associated with Th1-mediated cytokine production.³⁰ The pathogenesis of cAD has been shown to be associated with Th2 responses, although Th1 regulation was involved in chronic lesions.³¹ The study reported here has shown high IgG2 levels to antigens from both microbes in atopic dogs, even though an elevated IgG2 level is more consistent with nonatopic inflammatory diseases.⁹ This result implies involvement of a Th1 response to commensal microbes in cAD, but rigorous investigation of cytokines is still needed to help confirm this. In humans, immunity driven by T-helper 1 cytokines is required for protection from *Staphylococcus* spp. infection,³² whereas these cytokines promote inflammation in *Malassezia* spp. infections.³³ Given the complexity of immune responses shaped by different microbes, clarification is still needed regarding the bias of IgG subclass-associated T helper-cell polarization in dogs.

In the present study, the older dogs with cAD (Group 4) had a higher median CADLI score than the younger dogs (Group 2), although the difference was not statistically significant. However, no correlation was found between skin lesion severity and any of the specific antibodies to commensal microbes; this may reflect the complexity of the immune network in cAD, which involves many other factors such as keratinocyte-derived mediators, neuroimmune mediators and inflammatory cytokines that contribute to the itch-scratch cycle and promote skin inflammation. Based on the present results, detection of specific IgE to skin microbes should not be recommended as a biomarker for cAD or a gauge of its severity.

The increased levels of IgE specific to *M. pachydermatis* and *S. pseudintermedius* in dogs with cAD suggest a role for these organisms as allergens, but do not correlate with skin lesion severity as assessed by CADLI. These skin microbes also elicited different patterns of specific IgG subclass responses. The bias in IgG subclass relating to T helper-cell polarization requires further investigation in dogs.

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Supporting Information

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

Table S1. Demographic details of dogs in each group

Résumé

Contexte – Les taux élevés d’immunoglobulines E (IgE) à *Malassezia* et *Staphylococcus* dans la dermatite atopique de l’homme sont liés à un index de sévérité cutané; une association identique n’a pas été décrite chez le chien atopique.

Objectifs – Etudier les niveaux sériques d’IgE spécifiques d’allergènes, les IgG spécifiques totaux et les sous-classes d’IgG (IgG1 et IgG 2) pour *M. pachydermatis* et *S. pseudintermedius*, et de les relier à la sévérité de la dermatite du chien.

Sujets – Des échantillons sériques ont été prélevés de chiens catégorisés par âge et par statut médical. Les groupes 1 et 2 : <3 ans, respectivement sains (n = 9) et chiens atopiques (n = 9); et groupes 3 et 4: ≥3 ans, respectivement sains (n = 11) et atopiques (n = 14).

Matériels et méthodes – Les niveaux d’anticorps ont été mesurés par ELISA. Un CADLI (Canine Atopic Dermatitis Lesion Index) a été analysé en relation avec les taux d’anticorps.

Résultats – Les IgE spécifiques et les IgG totaux dirigés contre *M. pachydermatis* et *S. pseudintermedius* étaient significativement augmentés chez les chiens atopiques à tous les âges. Bien que des différences entre chiens atopiques et chiens sains, correspondant aux niveaux d'IgG 1 et IgG2 contre chaque microbe, variaient en importance selon les groupes d'âge. Aucun lien statistiquement significatif n'a été mis en évidence entre le CADLI et les niveaux d'immunoglobulines pour les deux types de microbes.

Conclusions et importance clinique – Dans la peau du chien, les microbes peuvent agir comme allergènes déclencheurs de réactions inflammatoires via les voies dépendantes d'IgE et IgG. L'affinité des sous classes d'IgG produites peuvent varier selon les types d'antigènes. Les niveaux spécifiques d'IgE peuvent être reliés au statut clinique chez le chien mais pas à la sévérité des lésions cutanées.

Resumen

Introducción – niveles elevados de inmunoglobulina E (IgE) para las especies de *Malassezia* o *Staphylococcus* en la dermatitis atópica humana están relacionados con el índice de gravedad de la piel; no se ha publicado una asociación similar en perros atópicos.

Objetivos – investigar los niveles séricos de IgE específica de alérgenos, niveles de IgG total y subclases de IgG específicas (IgG1 e IgG2) para *M. pachydermatis* y *S. pseudintermedius*, y correlacionarlas con la gravedad de la dermatitis en perros.

Animales – se obtuvieron muestras de suero de perros clasificados por edad y estado de la enfermedad. Grupos 1 y 2: <3 años de edad, sanos (n = 9) y perros atópicos (n = 9), respectivamente; y grupos 3 y 4: perros sanos ≥3 años (n = 11) y atópicos (n = 14), respectivamente.

Métodos y materiales – los niveles de anticuerpos se midieron por ELISA. El índice de lesión de la dermatitis atópica canina (CADLI) se analizó en relación con los niveles de anticuerpos.

Resultados – la IgE específica y la IgG total frente a *M. pachydermatis* y *S. pseudintermedius* aumentaron significativamente en perros atópicos de todas las edades. Aunque las diferencias entre perros atópicos y sanos, con respecto a los niveles específicos de IgG1 e IgG2 para cada microbio, variaron en importancia dentro de los grupos de edad. No se encontraron relaciones significativas entre el CADLI y los niveles de inmunoglobulina específicos para ambos tipos de microbios.

Conclusiones e importancia clínica – en la piel del perro, los microbios pueden actuar como alérgenos que desencadenan respuestas inflamatorias a través de vías dependientes de IgE e IgG. La afinidad de la subclase de IgG producida puede variar según el tipo de antígeno. Los niveles específicos de IgE pueden estar relacionados con la enfermedad clínica en perros y no con la gravedad de la lesión cutánea.

Zusammenfassung

Hintergrund – Erhöhte Immunglobulin E (IgE) Werte auf *Malassezia* und *Staphylococcus* Spezies bei der atopischen Dermatitis des Menschen stehen im Zusammenhang mit dem Skin Severity Index; bei Hunden konnte bisher kein ähnlicher Zusammenhang beschrieben werden.

Ziele – Eine Untersuchung von Serumwerten von Allergen-spezifischem IgE, Total spezifischem IgG und IgG Unterklassen (IgG1 und IgG2) auf *M. pachydermatis* und *S. pseudintermedius*, sowie eine Korrelation derselben mit dem Schweregrad der Dermatitis bei Hunden.

Tiere – Es wurden Serumproben von Hunden, die nach Alter und Krankheitsstatus eingeteilt wurden, genommen. Gruppen 1 und 2: < 3 Jahre alte gesunde (n=9) bzw atopische Hunde (n = 9); und Gruppen 3 und 4: ≥3 Jahre alte gesunde (n=11) bzw atopische Hunde (n =14).

Methoden und Materialien – Die Antikörperlevels wurden mittels ELISA gemessen. Der Canine Atopic Dermatitis Lesion Index (CADLI) wurde im Zusammenhang mit den Antikörperlevels analysiert.

Ergebnisse – Spezifische IgE und Total IgG auf *M. pachydermatis* und *S. pseudintermedius* waren bei atopischen Hunden allen Alters erhöht. Obwohl es Unterschiede gab zwischen atopischen und gesunden Hunden, in Bezug auf spezifische IgG1 und IgG2 Werte einer jeden Mikrobe, variierten diese in den unterschiedlichen Altersgruppen. Es wurden keine signifikanten Beziehungen zwischen den CADLI Werten und den jeweiligen spezifischen Immunglobulinlevels für beide Mikrobentypen gefunden.

Schlussfolgerungen und klinische Bedeutung – In der Hundehaut könnten Mikroben als Allergene wirken, die entzündliche Reaktionen über einen oder mehrere IgE – und IgG-abhängige Reaktionszyklen auslösen. Die Affinität der produzierten IgG Unterklasse kann in Bezug auf den Antigentyp variieren. Spezifische IgE Werte könnten mit der klinischen Erkrankung der Hunde im Zusammenhang stehen und nicht mit dem Schweregrad der Hautveränderungen.

要約

背景 – 人アトピー性皮膚炎におけるマラセチアまたはブドウ球菌種に対する免疫グロブリンE(IgE)レベルの上昇は、皮膚の重症度指数に関連している。しかし、同様の関連性はアトピー犬では報告されていない。

目的 – 本研究の目的は、アレルギー特異的IgEの血清レベル、*M. pachydermatis*および*S. pseudintermedius*の総特異的IgGおよびIgGサブクラス(IgG1およびIgG2)を調査し、それらを犬の皮膚炎重症度と関連させることである。

被験動物 – 血清サンプルを、年齢及び疾患状態によって分類された犬から収集した。グループ1および2:3歳未満の健康犬(n = 9)およびアトピー犬(n = 9)。グループ3および4:それぞれ3歳以上の健康犬(n = 11)およびアトピー犬(n = 14)。

材料と方法 – ELISAによって抗体レベルを測定した。犬アトピー性皮膚炎変数(CADLI)を抗体レベルとの関連について解析した。

結果 – *M. pachydermatis*および*S. pseudintermedius*に対する特異的IgEおよび総IgGは、全年齢層のアトピー犬で有意に増加した。各微生物に対する特異的IgG1およびIgG2レベルに関して、アトピー犬と健康犬の違いは、年齢層内で有意差があったことである。CADLIと両微生物の特異的免疫グロブリンレベル間に有意な関係は見られなかった。

結論と臨床的重要性 – 犬の皮膚において、微生物はIgEおよびIgG依存経路を介して炎症反応を誘発するアレルゲンとして作用する可能性がある。産生されるIgGサブクラスの親和性は、抗原の種類によって異なる場合がある。特定のIgEレベルは、犬の臨床疾患に関連している可能性があるが、皮膚病変の重症度に関連しているとは限らない。

摘要

背景 – 在人类异位性皮炎中,马拉色菌或葡萄球菌的免疫球蛋白E(IgE)水平升高与皮肤严重程度指数有关;类似的相关性在异位性犬中尚未报道。

目的 – 调查厚皮马拉色菌和假中间葡萄球菌的过敏原特异性IgE、总特异性IgG和IgG亚型(IgG1和IgG2)的血清水平,及其与犬异位性皮炎严重程度的相关性。

动物 – 按照年龄和疾病状况将犬进行分类,并收集血清样本。第1组和第2组:分别为<3岁的健康犬(n = 9)和异位性犬(n = 9);以及第3组和第4组:分别为≥3岁的健康犬(n = 11)和异位性犬(n = 14)。

方法和材料 – 通过ELISA检测抗体水平。分析犬异位性皮炎病变指数(CADLI)与抗体水平的关系。

结果 – 在各年龄段的异位性犬中,针对厚皮马拉色菌和假中间链球菌的特异性IgE和总IgG均显著增加。尽管异位性犬与健康犬的每种微生物特异性IgG1和IgG2水平存在差异,但在不同年龄组内显著性有所变化。CADLI与两种微生物的任何特异性免疫球蛋白水平之间,均未发现显著相关性。

结论和临床意义 – 在犬皮肤中,微生物可以通过IgE和IgG依赖性途径,作为过敏原触发炎症反应。产生的IgG亚型的亲和力可以根据抗原类型而变化。特异性IgE水平可能与犬的临床疾病有关,与皮肤病变的严重程度无关。

Resumo

Contexto – Os níveis elevados de imunoglobulina E (IgE) para espécies de *Malassezia* ou *Staphylococcus* na dermatite atópica humana estão relacionados ao índice de gravidade da pele; uma associação semelhante não foi relatada em cães atópicos.

Objetivos – Investigar os níveis séricos de IgE alérgeno-específica, IgG total e subclasses de IgG (IgG1 e IgG2) específicas para *M. pachydermatis* e *S. pseudintermedius* e correlacioná-los com a gravidade da dermatite em cães.

Animais – Foram coletadas amostras de soro de cães divididos por idade e status da doença. Grupos 1 e 2: cães saudáveis com <3 anos de idade (n = 9) e cães atópicos (n = 9), respectivamente; e grupos 3 e 4: ≥3 anos de idade, saudáveis (n = 11) e cães atópicos (n = 14), respectivamente.

Métodos e materiais – Os níveis de anticorpos foram mensurados por ELISA. O Índice de Lesão Dermatite Atópica Canina (*Canine Atopic Dermatitis Lesion Index* - CADLI) foi analisado em relação aos níveis de anticorpos.

Resultados – Houve aumento significativo de IgE específica e IgG total contra *M. pachydermatis* e *S. pseudintermedius* em cães atópicos de todas as idades. Embora as diferenças entre cães atópicos e saudáveis em relação aos níveis IgG1 e IgG2 específicas para cada microrganismo tenham variado significativamente dentro dos grupos etários, não foram encontradas relações significativas entre o CADLI e quaisquer níveis de imunoglobulinas específicas para ambos os tipos de microrganismos.

Conclusões e importância clínica – Na pele de cães, os microrganismos podem atuar como alérgenos, desencadeando respostas inflamatórias por vias dependentes de IgE e IgG. A afinidade da subclasse de IgG produzida pode variar de acordo com o tipo de antígeno. Níveis de IgE específica podem estar relacionados à doença clínica em cães e não à gravidade da lesão cutânea.

Supporting Information

Table S1 Demographic details of dogs in each group

Group	Number of dogs	Sex (Male: Female)	Median age and range (years)	Breed
1	9	5:4	1.67 (1.0-2.5)	Chihuahua (1); Labrador retriever (2); shiba inu (1); crossbreed (5)
2	9	4:5	1.58 (1.17-2.58)	American pitbull (1); beagle (2); bulldog (1); French bulldog (1); Labrador retriever (1); Pomeranian (1); West Highland white terrier (1); crossbreed (1)
3	11	7:4	3.85 (3.5-4.2)	Boxer (6); Rottweiler (3); Labrador retriever (1); crossbreed (1)
4	14	10:4	4.71 (3.5-6.0)	Bangkraw (1); beagle (1); Chihuahua (1); French bulldog (2); golden retriever (2); pug (1); Pomeranian (2); poodle (1); shih tzu (1); shiba inu (1); crossbreed (1)