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Expression of thymic stromal lymphopoietin in canine atopic dermatitis

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Background – In humans, thymic stromal lymphopoietin (TSLP) plays a central role in the development of allergic inflammation, such as atopic dermatitis (AD), but it is unknown whether it is involved in the pathogenesis of canine AD (CAD).

Hypothesis/Objectives – Our aim was to characterize canine *TSLP* and to assess its expression in CAD.

Methods – Canine *TSLP* was identified based on sequence homology with human *TSLP* and the complementary DNA (cDNA) cloned by RT-PCR. Real-time quantitative RT-PCR was established to assess the expression of canine *TSLP* in cultured canine keratinocytes and in skin biopsy specimens from lesional and nonlesional skin of 12 dogs with CAD and eight healthy control dogs.

Results – Partial canine *TSLP* cDNA was cloned and characterized. It contained four exons that shared 70 and 73% nucleotide identity with human and equine *TSLP*, respectively, encoding the signal peptide and full-length secreted protein. We found significantly increased *TSLP* expression in lesional and nonlesional skin of dogs with CAD compared with healthy control dogs ($P < 0.05$), whereas no difference was measured between lesional and nonlesional samples. In cultured primary canine keratinocytes, we found increased *TSLP* expression after stimulation with house dust mite allergen extract or Toll-like receptor ligands lipopolysaccharide and poly I:C.

Conclusions and clinical importance – Increased *TSLP* expression in the skin of dogs with CAD supports an involvement of TSLP in the pathogenesis of CAD similar to that in humans. Further studies should elucidate the function and therapeutic potential of TSLP in CAD.

Introduction

Thymic stromal lymphopoietin (TSLP) is an epithelial cell-derived cytokine, which plays a key role in differentiation of T-helper 2 (Th2) cells and development of allergic inflammation.[1] The expression of TSLP is increased in the skin of human patients with atopic dermatitis[2] and in the lungs of human asthma patients.[3] Results from mouse models support the central role of TSLP in allergic diseases, because tissue-specific overexpression of *TSLP* leads to the development of local allergic inflammation accompanied by a systemic Th2 response.[4,5] Conversely, mice lacking the TSLP receptor are more resistant to sensitization to inhaled allergens.[5] Thymic stromal lymphopoietin acts on dendritic cells (DCs), which then undergo maturation, express co-stimulatory molecules and efficiently present antigens.[2] In contrast to DCs stimulated by pathogens, human DCs stimulated by TSLP do not produce interleukin (IL)-12, which is the major T-helper 2 (Th1)-polarizing cytokine. Instead, TSLP-stimulated DCs induce differentiation of naive T cells into inflammatory Th2 cells, which produce IL-13, IL-4 and tumour necrosis factor-α.[2] Thus, TSLP may act as a master switch in allergic inflammation.[1]

Canine atopic dermatitis (CAD) is a common inflammatory skin disease of dogs, which shares several features with human atopic dermatitis (HAD), including predilection sites, genetic predisposition, age of onset, epidermal alterations[6] and immunopathological mechanisms.[7] Both HAD and CAD are characterized by imbalances in lymphocyte populations and their cytokines. Increased activities of both Th1 and Th2 cytokines have been observed in CAD, although results for individual cytokines differ among the studies. Nuttall *et al.*[8] observed overexpression of IL-4, interferon-γ, tumour necrosis factor-α and IL-2 and reduced expression of transforming growth factor-β1 in lesional skin of dogs with CAD. Another study[9] extended the analysis to include cytokines and transcription factors involved in T-helper polarization and showed increased expression of signal transducer and activator of transcription 4 (STAT4), IL-13, suppressor of cytokine signaling 3 (SOCS3) and IL-10 and decreased

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expression of IL-12p40 and GATA-3 in lesional skin. The Th2 cytokines, such as IL-4 and IL-13, drive production of allergen-specific IgE antibodies,[10] which are a central feature in the pathogenesis of CAD.[11] Epicutaneous allergen challenge of sensitized dogs leads to an early increase in the expression of IL-6 and IL-13.[12] In analogy to humans and mice, TSLP could play an important role in Th2 polarization in CAD. However, canine *TSLP* has not been characterized and its role in CAD is not known.

The aim of this study, therefore, was to identify and characterize the canine *TSLP* gene and to establish real-time quantitative RT-PCR (RT-qPCR) protocols to assess the expression of *TSLP* in lesional and nonlesional skin of dogs with CAD and in cultured canine keratinocytes.

Materials and methods

Animals

This study was performed using 12 dogs diagnosed with CAD (five females and seven males; age range 3–12 years, mean 6.25 years and median 6 years) and eight healthy control dogs (five females and three males; age range 1.5–10 years, mean 6.7 years and median 6 years) of the following breeds: Labrador retriever, golden retriever, beagle, boxer, Jack Russell terrier, German shepherd, Hovawart, bull terrier, German Wachtelhund, American cocker spaniel and Newfoundland. These dogs were used and characterized in a previous study.[6] The diagnosis of CAD was based on a combination of history and clinical signs,[13] with exclusion of other pruritic differential diagnoses. Skin biopsy specimens (8 mm) were obtained from both lesional and nonlesional skin in the dogs with CAD. Normal skin tissue was obtained from eight age-matched and, if possible, breed-matched dogs with no known history or clinical signs of CAD. These dogs were euthanized for reasons unrelated to our study, and samples were taken immediately after euthanasia. The study protocol was approved by the local ethical committee on the use of animals, Canton Bern, Switzerland (BVET 84/05).

Isolation and culture of primary canine keratinocytes

Canine footpad keratinocytes were isolated from 10-week-old beagle dogs and cultured in monolayers as described.[14,15] Cells were maintained in William's E medium (BioConcept, Allschwill, Switzerland) including antibiotics (Gibco Antibiotic-Antimycotic 100X liquid; Gibco, Invitrogen, Carlsbad, CA, USA), 10% fetal calf serum (Gibco), 2 mmol/L L-glutamine (Gibco), 10^{-10} mol/L cholera toxin (Sigma-Aldrich, St Louis, MO, USA) and 10 ng/mL of human epidermal growth factor (EGF, E9644; Sigma-Aldrich). Monolayer culture cells were incubated at 34°C in air supplemented with 5% CO_2, passaged after trypsin digestion (5× Trypsin/EDTA PBS; BioConcept) and subcultured before they reached confluence. Cells between passages 12 and 20 were used for these experiments. For stimulation experiments, keratinocytes were incubated in complete medium lacking cholera toxin. Keratinocytes were stimulated for 4 h with phorbol 12-myristate 13-acetate (PMA, 50 ng/mL; Sigma-Aldrich) and ionomycin (1 μmol/L; Sigma-Aldrich) and for 18 h with concanavalin A (ConA, 5 μg/mL; Sigma-Aldrich), the Toll-like receptor (TLR)-4 ligand lipopolycaccharide (LPS, 1 μg/mL, derived from *Esherichia coli* 0128:B12; Sigma-Aldrich), the TLR-3 ligand polyinosinic:polycytidylic acid (poly I:C, 5 μg/mL; Sigma-Aldrich), the TLR-7 ligand imiquimod (5 μg/mL; Invivogen, San Diego, CA, USA), the TLR-2 ligand Pam3Cys (10 μg/mL; EMC microcollections, Tübingen, Germany), house dust mite extract (HDM, *Dermatophagoides farinae*, 5 μg/mL; Heska, Fribourg, Switzerland) and heat-inactivated HDM (5 μg/mL). Heat inactivation was performed at 99°C for 10 min in order to inhibit enzymatic activity of HDM. The TLR ligands were used at concentrations previously shown to induce activation of canine dendritic cells.[16] Cells were lysed in RLT buffer (Qiagen, Basel, Switzerland), and RNA was isolated as described in the next subsection.

Isolation of RNA and synthesis of cDNA

Total RNA was isolated from cultured keratinocytes using RNeasy Mini Kit (Qiagen) and from skin biopsy specimens using RNeasy Fibrous Tissue Kit (Qiagen) according to the manufacturer's instructions. Contaminating genomic DNA was removed by on-column DNase treatment. Total RNA was quantified spectrophotometrically at 260 nm (NanoDrop 1000; Thermo Scientific, Reinach, Switzerland), and samples were stored at –80°C until used.

Reverse transcription of total RNA was performed using Superscript III RT (Invitrogen) to generate complementary DNA (cDNA), which was used as a template for PCR. The cDNA synthesis reaction consisted of 500 ng RNA and 1 mmol/L random primers (Promega, Dübendorf, Switzerland), 0.5 mmol/L of each dNTP, 40 U RNase inhibitor (Promega) and 100 U SuperScript III reverse transcriptase (Invitrogen), in a total reaction volume of 40 μL. The RNA samples, random primers and dNTPs were incubated at 65°C for 5 min. Reverse transcriptase was added and the incubation continued at 25°C for 5 min followed by 50°C for 50 min. The reaction was terminated by heating at 70°C for 15 min. The cDNA samples were stored at –20°C until further processing.

Cloning of canine *TSLP*

To clone the canine *TSLP* cDNA, sequences homologous to human *TSLP* (GenBank accession NM_033035.4) were identified in *Canis familiaris* genome build 3.2 using BLASTN (blast.ncbi.nih.gov). From a skin biopsy specimen obtained from a dog affected with CAD, RNA was isolated and cDNA produced. Primers were designed using Primer3[17] based on homologies with human and equine[18] (Genbank NM_001164063) *TSLP*. The cloned primary sequence was determined by direct sequencing. Alignment of human and canine *TSLP* nucleotide and deduced amino acid sequences was performed using ClustalW.[19]

Real-time quantitative RT-PCR

Expression of *TSLP* mRNA was assessed by RT-qPCR with primers designed to span the exon 1 and 2 junction using Primer3 online software[16] (forward, 5′-AGT ACA CGG GGT GGC TGA-3′; reverse, 5′-GTC ATT TAC CAA GCC CTG GA-3′; and probe, 5′-FAM-TCC GTG CTC CTG GTC CCA TCC ATG TAT T-TAMRA-3′). The PCR consisted of 2 μL cDNA, primers (300 nmol/L each), fluorescence-labelled probe (200 nmol/L) and TaqMan Universal PCR Mastermix (Applied Biosystems, Austin, TX, USA). The PCRs were performed in a total volume of 25 μL and carried out in a 7300 Real-Time PCR System (Applied Biosystems). The PCR consisted of an initial 2 min at 50°C, 10 min denaturation at 95°C, followed by 45 cycles of 15 s denaturation at 95°C and 1 min annealing and elongation at 60°C. Reactions were performed in duplicates, and no-template and RT-negative controls were included in each run. Efficiencies of the PCRs were calculated using a relative standard curve derived from a cDNA pool. Gene expression was quantified using the $\Delta\Delta Ct$ method.[20] Expression of *TSLP* was normalized to the expression of the housekeeping gene 18S ribosomal RNA (18S rRNA; Applied Biosystems).

Statistical analysis

Statistical analyses were carried out with the software package NCSS 2001 (NCSS, Kaysville, UT, USA). The Mann–Whitney *U*-test was used to compare dogs withCAD with healthy control dogs. The Wilcoxon signed rank test was used for comparison of paired data (lesional versus nonlesional). Student's two-sample *t* test (unpaired) was used to compare stimulated and nonstimulated keratinocytes.

Results

Characterization of canine *TSLP*

A partial cDNA of canine *TSLP* was generated by RT-PCR from an RNA sample of lesional skin from a dog with CAD, cloned and sequenced and the sequence

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deposited in GenBank (accession JQ698664). The cDNA sequence contained 465 nucleotides and was organized in four exons (Figure 1). The exon–intron boundaries were in accord with the splicing scheme for human *TSLP* gene reported previously by Quentmeier *et al.*,[21] whose numbering scheme was followed by the investigators. The splice donor and acceptor sites were consistent with the GT/AG rule. The canine *TSLP* gene was located on chromosome 3 (1.503–1.507 Mbp), which corresponds to human chromosome 5 and equine chromosome 14. When the coding sequence of canine *TSLP* cDNA was aligned with the orthologous human and equine sequences (GenBank accession numbers NM_033035.4 and NM_001164063.1), the nucleotide identity was 70 and 73%, respectively, and covered the sequence encoding the signal peptide and full-length mature protein of human and equine *TSLP*. Alignment of canine and human nucleotide sequences is shown in Figure 2a.

The predicted amino acid sequence deduced from the canine *TSLP* cDNA shares 60.8 and 59.9% identity with human (NP_149024.1) and equine TSLP protein (NP_001157535.1), respectively. Alignment between canine and human TSLP proteins is shown in Figure 2b. Although the N-terminal part of the protein was not determined, it was possible to predict the cleavage site of the putative signal peptide and the N-terminus of the mature protein (Figure 1; SignalP, http://www.cbs.dtu.dk/services/SignalP), which are analogous to human and equine TSLP proteins. Furthermore, the deduced amino acid sequence ended with a stop codon, suggesting that the partial cDNA encodes the signal peptide and mature canine TSLP protein (Figure 1).

Increased expression of *TSLP* in the skin of dogs with atopic dermatitis

The expression of *TSLP* mRNA was determined by RT-qPCR in lesional and nonlesional skin of the 12 dogs with CAD and compared with skin from the eight healthy control dogs. The expression of *TSLP* was significantly higher in both lesional and nonlesional skin of dogs with CAD compared with the control dogs ($P < 0.05$; Figure 3), but there was no significant difference between lesional and nonlesional samples.

Expression of *TSLP* in primary canine keratinocytes

The primary canine keratinocyte cell line generated from canine footpad was stimulated with PMA + ionomycin, TLR ligands (LPS, polyI:C, Pam3Cys and imiquimod) and HDM, and the expression of *TSLP* was measured by RT-qPCR (Figure 4). The PMA + ionomycin induced the strongest upregulation of *TSLP* expression (262-fold increase; $P < 0.05$).

Lipopolysaccharide and poly I:C also upregulated *TSLP* expression in canine keratinocytes (15.9- and 18.1-fold increase, respectively; $P < 0.05$), whereas the other TLR ligands had no significant effect on *TSLP* expression. Concanavalin A also failed to induce *TSLP* expression in canine keratinocytes. Keratinocytes stimulated with HDM responded with upregulated *TSLP* expression (27-fold increase; $P < 0.05$). The effect of HDM was inhibited by heat inactivation of HDM at 99°C, i.e. the expression of TSLP was significantly lower after stimulation with heat-inactivated HDM than with intact HDM ($P < 0.05$; Figure 4, indicated by #).

Discussion

In the present study, we identified and characterized canine *TSLP* and assessed the expression of *TSLP* in the skin of dogs with CAD and in cultured primary canine keratinocytes. We found that *TSLP* expression was increased in both lesional and nonlesional skin samples from dogs with CAD compared with healthy control dogs (Figure 3). Moreover, we observed that primary canine keratinocytes upregulated *TSLP* expression when stimulated with HDM allergen extract (Figure 4). These findings suggest that TSLP may play an important role in the pathogenesis of CAD.

We identified and amplified a partial *TSLP* sequence analogous to human and equine *TSLP* that covers the signal peptide and full-length mature protein. There is a predicted canine *TSLP* sequence in GenBank (accession XM_844431.2), but when we designed primers based on this sequence we were not able to amplify it by RT-PCR. A possible explanation is that this sequence might be faulty, or it encodes a different gene or splicing variant that is not expressed in the tissue examined. Although our *TSLP* sequence does not cover the entire

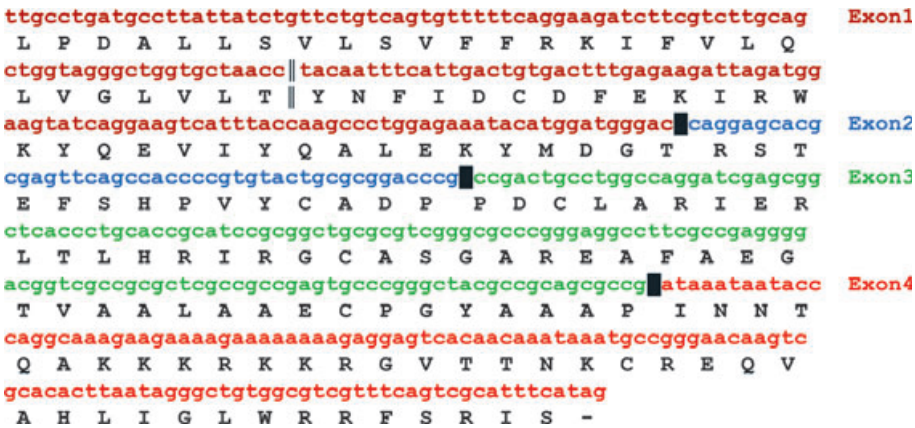

Figure 1. Canine thymic stromal lymphopoietin (*TSLP*) nucleotide sequence and the deduced amino acid sequence. Exon–exon boundaries are marked by filled squares. The predicted signal peptide cleavage site is marked by ‖.

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Figure 2. Alignment of canine and human *TSLP* nucleotide (a) and amino acid sequences (b) performed by ClustalW. Identities are shown by '*' and similarities by ':' and '.'. Start and stop codons are underlined.

coding sequence of canine *TSLP* (the 5′-terminal part is missing), it is possible to predict the signal peptide cleavage site, which is well conserved among species. Therefore, the sequence coding for the full-length mature TSLP protein is included in our sequence and it will be sufficient for cloning and expressing of recombinant canine TSLP protein, which will be needed for further functional studies and for production of canine TSLP-specific antibodies.

We established RT-qPCR and used it to assess the expression of *TSLP* in skin biopsy samples and in cultured keratinocytes. While RT-qPCR is a very sensitive and accurate method, its major limitation is that it does not allow identification of cellular sources of TSLP in tissue samples and does not assess the presence or biological activity of TSLP protein. In order to address these important questions, tools need to be developed, such as antibodies for immunohistochemistry or enzyme-linked immunosorbent assay.

The *TSLP* expression was measured in a small group of healthy dogs and CAD-affected dogs of various breeds. To confirm our findings, a larger study involving more dogs needs to be completed and expression of other genes assessed. A larger group of dogs would allow the association between *TSLP* expression and severity of lesions to be assessed. To gain more insight into the mechanisms of action of TSLP, the expression of TSLP target genes, such as *IL-4*, *IL-13*, *OX40L*, macrophage-derived chemokine (MDC) and thymus and activation-regulated chemokine (TARC)[2] needs to be measured.

A previous study using the same population of dogs has shown an aberrant expression of filaggrin in the skin of some dogs with CAD.[6] An epidermal barrier defect, aberrant filaggrin expression and increased TSLP expression may all play an important role in the pathogenesis of CAD, because a similar mechanism has been proposed in human AD.[22]

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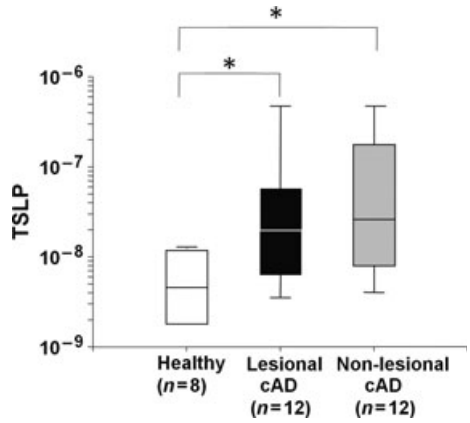

Figure 3. Expression of *TSLP* in canine atopic dermatitis (CAD). Relative expression of canine *TSLP* in lesional and nonlesional skin of 12 dogs with CAD and eight healthy control dogs. Values are normalized to the expression of a housekeeping gene, 18S rRNA. Statistical analysis was performed using the Mann–Whitney *U*-test (* $P < 0.05$).

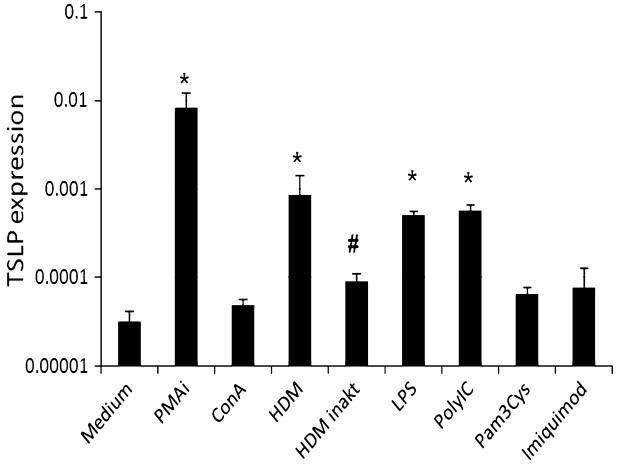

Figure 4. Expression of *TSLP* in canine keratinocytes. Relative expression of canine *TSLP* in cultured primary canine keratinocytes incubated with medium alone, or stimulated with phorbol 12-myristate 13-acetate (PMA, 50 ng/mL) + ionomycin (1 μmol/L; PMAi), concanavalin A (ConA, 5 μg/mL), lipopolysaccharide (LPS, 1 μg/mL), polyinosinic:polycytidylic acid (poly I:C, 5 μg/mL), imiquimod (5 μg/mL), Pam3Cys (10 μg/mL), house dust mite extract (HDM, 5 μg/mL) or heat-inactivated HDM (HDM inact, 5 μg/mL). Statistical analysis was performed by Student's two-sample *t*-test (unpaired). Significant differences between stimulants and medium are shown by * ($P < 0.05$); difference between HDM and heat-inactivated HDM is indicated by # ($P < 0.05$).

Stimulation of canine keratinocytes with HDM resulted in increased expression of *TSLP*. This finding highlights the role of TSLP in CAD, because HDM contains major allergens involved in the pathogenesis of CAD. Heat inactivation of HDM abrogated the induction of *TSLP* (Figure 4), suggesting that proteases or other heat-labile components are responsible for the induction of *TSLP* expression. Protease allergens have been shown to induce *TSLP* expression in human epithelial cells through the activation of protease-activated receptor-2.[23] Several mechanisms have been proposed by which proteases may facilitate sensitization and priming of Th2 responses.[24] Through the disruption of tight junctions, proteases may facilitate the transepithelial transfer of allergens. Proteases may also change the activity of DCs by cleaving of the co-stimulatory molecules CD40[25] or DC-SIGN,[26] as has been shown for Der p 1 allergen. Allergen proteases may also directly influence adaptive immune responses by cleaving of CD25 on T cells or CD23 on B cells. Cleavage of CD25 (IL-2 receptor α-chain) by Der p 1 resulted in reduced Th1 and enhanced Th2 cytokine expression in cultured T cells.[27] Der p 1 can also cleave CD23, the low-affinity receptor for IgE, which normally acts as a negative regulator of IgE synthesis.[28] More detailed studies will be needed to dissect the potential role of allergen proteases in CAD.

We used whole-body HDM extract for the stimulation of canine keratinocytes in our study. As this also includes mite gut content, it is likely to contain microbial molecules (TLR ligands) in addition to allergens. Therefore, the effect of HDM on *TSLP* expression measured in our study may be mediated, at least in part, by TLR ligands. We also included stimulation with purified TLR ligands and found that LPS and poly I:C, but not Pam3Cys or imiquimod, induced *TSLP* expression in cultured canine keratinocytes (Figure 4). It has been shown previously that keratinocytes express functional TLRs and can respond to TLR stimulation by production of cytokines,[29] including TSLP.[30] However, it is still unclear how TLR stimulation contributes to allergic responses.

Genetic analysis provides additional evidence of the involvement of TSLP in CAD. Genotyping of single nucleotide polymorphisms in 25 candidate genes in dogs of various breeds revealed an association between a single nucleotide polymorphism in the TSLP receptor gene and CAD in all studied breeds.[31] In human patients, associations between allergic diseases and polymorphisms of the *TSLP* gene have been described.[32,33]

In conclusion, we characterized the canine *TSLP* gene and found increased expression of *TSLP* in the skin of dogs with atopic dermatitis. These preliminary findings indicate an involvement of TSLP in the pathogenesis of atopic dermatitis in dogs. Further studies should elucidate the function of TSLP in CAD and explore its potential as a therapeutic target.

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Résumé

Contexte – Chez l'homme, la TSLP (thymic stromal lymphopoietin) joue un rôle central dans le développement de l'inflammation allergique, telle que la dermatite atopique (AD), mais nous ne savons pas si elle est impliquée dans la pathogénie de la dermatite atopique canine (CAD).

Hypothèse / objectifs – Notre objectif est de caractériser la TSLP canine et d'évaluer son expression dans la CAD.

Méthodes – La TSLP canine a été identifiée par homologie de séquence avec la TSLP humaine et de l'ADN complémentaire (ADNc) clonée par RT-PCR. La RT-PCR quantitative en temps réel a été établie afin d'évaluer l'expression de la TSLP canine dans les kératinocytes canins en culture et dans les biopsies de peaux lésionnelles et non lésionnelles de 12 chiens avec CAD et huit chiens de contrôle sains.

Résultats – L'ADNc partielle de TSLP canine a été cloné et caractérisé. Il contient quatre exons qui partagent respectivement 70 et 73 % d'identité nucléotidique avec la TSLP humaine et équine, codant pour le peptide signal et la protéine sécrétée intégrale. Nous avons trouvé une expression de TSLP significativement accrue dans la peau lésionnelle et non-lésionnelle des chiens avec CAD par rapport aux chiens témoins sains ($P < 0.05$), alors qu'aucune différence n'a été mesurée entre les échantillons lésionnels et non-lésionnels. Dans les kératinocytes canins primaires en culture, nous avons trouvé une expression de

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TSLP accrue après stimulation avec les extraits d'allergènes d'acariens de poussière de maison ou les lipopolysaccharides ligands du Toll-like recepteur et poly I:C.
Conclusions et importance clinique – L'expression augmentée de TSLP dans la peau des chiens avec CAD supporte la participation de la TSLP dans la pathogénie de la CAD, semblable à celle de l'homme. De plus amples études devraient élucider la fonction et le potentiel thérapeutique de la TSLP dans la dermatite atopique canine.

Resumen
Introducción – en seres humanos, la linfopoietina tímica del estroma (TSLP) juega un papel central en el desarrollo de la inflamación alérgica, como en dermatitis atópica (AD), pero se desconoce si juega un papel en la patogenia de la AD canina (CAD).
Objetivos del / de la hipótesis – nuestro propósito fue caracterizar la *TSLP* canina y determinar su expresión en casos de CAD.
Métodos – se identificó la *TSLP* canina basado en la homología de la secuencia con la *TSLP* humana y el DNA complementario (cDNA) fue clonado mediante RT-PCR. Se preparó RT-PCR cuantitativa en tiempo real para determinar la expresión de la *TSLP* canina en queratinocitos caninos cultivados y en biopsias de piel con lesiones y sin lesiones de 12 perros con CAD y de ocho perros sanos como control.
Resultados – se clonó y caracterizó parcialmente el cDNA de la *TSLP*. Tenia cuatro exones que compartieron un 70 y un 73% de homologia en nucleótidos con la *TSLP* humana y equina, respectivamente, codificando el péptido de señal y la proteína secretada completa. Encontramos una expresión mayor de TSLP en la piel con y sin lesiones de perros con CAD comparado con los perros sanos control ($P < 0,05$), mientras que no se observó ninguna diferencia entre las muestras con y sin lesiones. En queratinocitos caninos primarios cultivados, encontramos una expresión creciente de la *TSLP* después del estímulo con el extracto alergénico del ácaro del polvo de la casa o con los ligandos de los receptores equivalentes a Toll lipopolisacarido y poli I:C.
Conclusiones e importancia clínica – la expresión creciente de *TSLP* en la piel de perros con CAD sugiere un papel de la *TSLP* en la patogenia de la CAD, similar a lo que sucede en seres humanos. Otros estudios deben aclarar la función y el potencial terapéutico de la TSLP en casos de CAD.

Zusammenfassung
Hintergrund – Beim Menschen spielt das thymische stromale Lymphopoietin (TSLP) eine zentrale Rolle bei der Entwicklung allergischer Entzündung, wie zum Beispiel bei atopischer Dermatitis (AD), aber es ist nicht bekannt, ob es auch bei der Pathogenese der caninen AD (CAD) eine Rolle spielt.
Hypothese /Ziele – Unser Ziel war eine Beschreibung des caninen *TSLP* vorzunehmen und seine Exprimierung bei der CAD festzustellen.
Methoden – Canines *TSLP* wurde mittels Sequenzhomologie mit humanem *TSLP* identifiziert und die komplementäre DNA (cDNA) mittels RT-PCR geklont. Ein Real-time quantitativer RT-PCR wurde entwickelt, um die Exprimierung des caninen TSLP in kultivierten caninen Keratinozyten und in Proben aus Hautbiopsien läsionaler und nicht-läsionaler Haut von 12 Hunden mit CAD und acht gesunden Kontrollhunden zu erfassen.
Ergebnisse – Eine partielle *TSLP* cDNA wurde geklont und charakterisiert. Dieser Teil beinhaltete vier Exons, welche mit dem humanen *TSLP* zu 70% und mit dem equinen *TSLP* zu 73% ident waren und das Signalpeptid und das sezernierte Protein in voller Länge kodierten. Wir stellten eine signifikant erhöhte *TSLP* Exprimierung in läsionaler und nicht-läsionaler Haut von Hunden mit CAD fest im Vergleich zu gesunden Kontrollhunden ($P < 0,05$), während wir zwischen Proben aus Läsionen und normaler Haut keine Unterschiede feststellen konnten. In kultivierten primären caninen Keratinozyten fanden wir eine erhöhte TSLP Exprimierung nach einer Stimulation mit Hausstaubmilbenextrakt oder Lipopolysacchariden der Toll-like Rezeptorliganden und Poly I:C.
Schlussfolgerungen und klinische Bedeutung – Eine erhöhte *TSLP* Exprimierung in der Haut von Hunden mit CAD unterstützt die Hypothese der Beteiligung von *TSLP* bei der Pathogenese von CAD, ähnlich wie beim Menschen. Weitere Studien sollten das funktionelle und therapeutische Potential von TSLP bei der CAD beleuchten.

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要約

背景–ヒトでは、胸腺間質性リンパ球新生因子（TSLP）はアトピー性皮膚炎(AD)などのアレルギー性炎症の発症における中心的な役割を担っているが、犬アトピー性皮膚炎（CAD）の病因に対する関与は明らかではない。

仮説/目的–筆者らの目的はイヌ*TSLP*を明らかにすることと、CADでの発現を評価することである。

方法–イヌ*TSLP*はヒト*TSLP*との配列の相同性に基づいて特定し、相補的DNA（c DNA）はRT-PCRにてクローニングした。イヌ*TSLP*の発現を評価するために，培養イヌケラチノサイトならびに12頭のCADの病変部と非病変部皮膚，および8頭の健康な対照犬の皮膚を用いてリアルタイム定量的PCRを実施した。

結果–イヌ*TSLP* c DNAの一部をクローニングし、解析した。この遺伝子は4つのエクソンからなり、塩基配列はヒトとウマ*TSLP*と、それぞれ70%と73%の相同性を示しており、シグナルペプチドと完全長の分泌タンパクをコードしていた。筆者らは健康な対照犬と比較し、CADの病変部と非病変部での*TSLP*発現が有意に増加（$P < 0.05$）していたことを観察した一方、病変部と非病変部では変化はなかった。培養した初代イヌケラチノサイトでは、筆者らはハウスダストマイトアレルゲン抽出物、Toll様受容体リガンドであるリポ多糖、ポリI:Cで刺激した後、*TSLP*の発現は増加したことを見いだした。

結論と臨床的な重要性–CADのイヌの皮膚での*TSLP*発現の増加はヒトと同様にCADの病因に*TSLP*が関与していることを支持した。さらなる研究が機能とCADでのTSLPの治療への可能性の解明に必要である。

摘要

背景–胸腺基质淋巴生成素(TSLP)在人过敏性炎症，例如异位性皮炎的发生中起核心作用，但是尚不知犬AD的发病机制谁否与此因素有关。

假设/目的–我们的目的是找到犬TSLP的特点，并评价在CAD中的表达。

方法–犬*TSLP*的鉴定基于人*TSLP*的序列同源性，并由RT-PCR克隆互补DNA（cDNA）。选取培养的犬角质形成细胞，以及12只AD患犬和8只健康对照犬的病变和非病变皮肤活检样本，建立实时定量RT-PCR来评估上述样本中犬*TSLP*的表达。

结果– 克隆和描述部分犬的*TSLP* cDNA的特征。有4种外显子编码信号肽和全长分泌蛋白，这些外显子与70%人和73%马*TSLP*的核酸序列相同。我们发现犬AD病变和非病变皮肤相比于健康犬有明显增多的*TSLP*表达（$P < 0.05$），而病变和非病变的样本测试没有发现不同。我们发现尘螨抗原提取物或者Toll样受体的配体脂多糖和多聚I:C刺激，使培养的初级犬角质形成细胞TSLP表达增高。

结论和临床价值–AD患犬皮肤的TSLP表达增多，支持了CAD与人的发病机制相似的理论。而且研究应该阐明TSLP的功能以及在CAD中的治疗潜力。

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