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The effects of the canine type C staphylococcal enterotoxin on the proliferation and cytokine expression of peripheral blood mononuclear cells from atopic and healthy dogs

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Abstract

Canine atopic dermatitis (AD) is defined as a genetically predisposed inflammatory and

pruritic allergic skin disease associated with skin colonization by *Staphylococcus pseudintermedius* known to produce exotoxins with superantigen activity. Despite the high prevalence of the canine type C staphylococcal enterotoxin (SEC_{canine}) in *S. pseudintermedius* of canine origin, its significance in *S. pseudintermedius* infections has not been well investigated. In this pilot study, the effects of SEC_{canine} on proliferation and cytokine responses in canine peripheral blood mononuclear cells (PBMC) were investigated. PBMC from seven atopic dogs and six healthy dogs were stimulated with SEC_{canine} and PBMC proliferation was demonstrated using WST-1 assay and the expression levels of IL-4, IL-5, IL-10, IL-13, IFN-γ, and TNF-α mRNAs in PBMC were quantified using a real-time PCR. Low concentration of SEC_{canine} induced potent T cell proliferation in both atopic and normal dogs. PBMC of atopic dogs appear to be less substantial responses to SEC_{canine} than those of the healthy controls. Of all the cytokines investigated in PBMC, the expression level of IL-4, IL-13, and IFN-γ mRNA was increased in both groups. Interestingly, the expression level of IFN-γ mRNA in atopic

dogs was significantly higher than that in the healthy dogs. The results indicate that less

proliferative response of PBMC than healthy dogs and Th1 like cytokine response occur

in response to SEC$_{canine}$ in atopic dogs. These findings suggest that an infection of *S.*

pseudintermedius producing SEC$_{canine}$ may play a role in aggravation factor of atopic

dermatitis by reducing proliferation in T cells and immune response of canine AD by

facilitating the development of Th1 cell dominated chronic lesion.

CONTENTS

Introduction

Atopic dermatitis (AD) is a common inflammatory skin disease of humans (Rothe and Grant-Kels 1996) and dogs (Scott, Miller et al. 2001). It has been defined as a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features associated with IgE antibodies, most commonly directed against environmental allergens (Halliwell 2006).

Staphylococcal infection is the major complicating factor in AD (Olivry, DeBoer et al. 2001). A normal inhabitant of the skin and mucosa of dogs, *Staphylococcus pseudintermedius*, is the major bacterial pathogen known to be associated with canine AD (Bannoehr and Guardabassi 2012). *S. pseudintermedius* produces a range of exotoxins, including staphylococcal enterotoxins (SEs) with superantigenic properties. The role of superantigens has been studied extensively in human and mice (Woodland, Wen et al. 1997, Chatila, Scholl et al. 2010). Interactions between T cell receptors, superantigens and major histocompatibility complex (MHC) class II molecules may lead to strong T cell activation, anergy, apoptosis or massive cytokine secretion (Fleischer 1994, Webb and Gascoigne 1994, Miethke, Wahl et al. 1995). Abnormal function of T-lymphocytes has been reported in both atopic dogs (Nimmo Wilkie, Yager et al. 1991) and people (Akdis, Trautmann et al. 2001). In human AD, it is currently accepted that imbalances in lymphocyte populations and cytokine production play an important role in the pathogenesis of the disease (Akdis, Trautmann et al. 2001, Olesen 2001).

Given the immunological similarities with human AD, it was proposed that staphylococcal superantigens produced by canine staphylococcal isolates might play a pathogenic role in canine AD as well (Marsella and Olivry 2003). This study was specifically investigated the role of the canine type C SE (SEC$_{canine}$) which is one of the SEC variants (SEC1, SEC2, SEC3, SEC$_{ovine}$ and SEC$_{bovine}$). Previous studies reported a high prevalence of SEC$_{canine}$ in *S. pseudintermedius* of canine origin (Edwards, Deringer et al. 1997, Hendricks, Schuberth et al. 2002, Yoon, Lee et al. 2010) and demonstrated its superantigenic activity such as an emetic response and T lymphocyte proliferation (Edwards, Deringer et al. 1997, Hendricks, Schuberth et al. 2002). Especially, the recent study reported that the frequency of SEC$_{canine}$ isolation was significantly higher in atopic dogs than in healthy dogs (Eui-Hwa Nam 2013). However, its biological significance of *S. pseudintermedius* infections has been rarely studied.

The present study was aimed to investigate the role of staphylococcal superantigens in the pathogenesis of canine AD by analyzing the proliferative potential and production of cytokines in response to SEC$_{canine}$ in peripheral blood mononuclear cells (PBMC) from atopic and healthy dogs.

Materials and Methods

1. Study population

Seven dogs with AD presented the Veterinary Medical Teaching Hospital of Seoul National University, The Republic of Korea, were included in this study. Diagnosis was made based on compatible history, clinical findings and positive results of intradermal skin test and/or serology allergen specific IgE test, with exclusion of other differential diagnoses for pruritus including adverse food reactions and ectoparasite infestation. The age of the dogs ranged from 1 to 10 years with an average age of 5.8 years. The breeds of the dogs included Shih Tzu, Bulldog, American Cocker Spaniel, Beagle, and Cavalier King Charles Spaniel (Table 1). Anti-inflammatory medications were withdrawn at least 3 weeks prior to the intradermal skin test, serology allergen specific IgE test, and blood collection, according to the published study (Olivry and Saridomichelakis 2013). Six healthy beagle dogs with an average age of 2.3 years with no history or clinical signs of skin diseases were used as controls (Table 1).

2. Isolation and culture of PBMC

PBMCs were prepared by density gradient centrifugation of citrate phosphate dextrose adenine (CPDA-1)-treated peripheral blood samples obtained from atopic and healthy dogs. A volume of peripheral blood was diluted with an equal

volume of PBS, layered onto Histopaque 1077 (Sigma-Aldrich, MO, USA), and centrifuged at 440 g for 30 min at room temperature. Cells were collected from the interface, washed in PBS and the residual erythrocytes were lysed by red blood cell lysis buffer (8.3 g/L ammonium chloride and 0.01 M Tris-HCl buffer, pH 7.5; Sigma-Aldrich). Following washing, PBMC were suspended in complete medium consisting of RPMI 1640 medium (Gibco BRL, Gaithersburg, MD, USA) supplemented with 10% heat-inactivated fetal bovine serum (Hyclone), 100 U/ml penicillin and 100 µg/ml streptomycin (Gibco BRL). Cell viability was 95-100% as determined by trypan blue exclusion.

3. Proliferation assay

PBMCs (2×10^5 cells/well) were cultured in culture media added with phytohaemagglutinin (PHA; 5 µg/ml; Sigma), SEC_{canine} (0.01-5 ng/ml) or culture medium alone in 96-well microtiter plates (SPL Life Science, Seoul, Korea) separately. Cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2. After 72 h incubation, 10 µl of EZ-Cytox cell viability assay kit solution (WST-1^{TM}; Daeil Lab Service, Seoul, Korea) was added and incubated for additional 4 h. Absorbance was measured at 450 nm with reference wavelength at 655 nm by ELISA reader (Bio-Rad, Munich, Germany). The experiments were carried out in triplicate. Proliferation was expressed as the stimulation index (SI) using the formula $(S - C)/C \times 100$, with S as the absorbance of stimulated cells and C as the absorbance of control unstimulated cells.

4. Cytokine expression in PBMC after treatment with SEC$_{canine}$

PBMCs (6 x 10^6 cells/well) were stimulated in 6 well plates (SPL Life Science) with or without SEC$_{canine}$ (0.1 ng/ml) for 24 h at 37°C in a humidified atmosphere containing 5% CO$_2$. Based on a preliminary study, the optimal concentration of SEC$_{canine}$ and incubation period were determined by measuring the expression of cytokine mRNA based on our preliminary study. After stimulation, total RNA was extracted from the cultured PBMCs using the Hybrid-R^{TM} kit (GeneAll Biotechnology, Seoul, Korea) according to the manufacturer's instructions. cDNA was synthesized from the total RNA using PrimeScript First Strand cDNA synthesis kit (TaKaRa, Tokyo, Japan).

Quantification of cytokine expression was accomplished with a StepOne Plus Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) using SYBR Premix Ex Taq II (TaKaRa). The sequences of the primers for each cytokine used in this study are shown in Table 2. Thermal cycling conditions were 2 min at 50°C and 10 min at 95°C, followed by 45 cycles of 15 sec at 90°C and 1 min at 60°C. Results of cytokine expression were represented as cycle threshold (Ct) values, which were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal control. The relative expression for each cytokine was reported as the n-fold difference in the normalized target gene expression level between stimulated and unstimulated sample.

5. Statistical analysis

Statistical comparisons of proliferation and cytokine expression between 2 groups were performed with the Mann-Whitney U-test. Data were expressed as mean ± standard deviation (SD). A value of P < 0.05 was considered significant.

Results

1. PBMC proliferative response to SEC$_{canine}$

The effect of SEC$_{canine}$ on the proliferation of PBMC were evaluated by WST-1 assay based on the ability of viable cells to cleave tetrazolium salts by mitochondrial dehydrogenase. After 72 hours of incubation in the presence of SEC$_{canine}$, there was evidence of PBMC proliferation compared with unstimulated groups in both atopic dogs and healthy dogs. Table 3 shows the results of proliferation to a range of concentrations of SEC$_{canine}$ in PBMC. Low concentration (0.01 and 0.1 ng/ml) of SEC$_{canine}$ induced more number of PBMCs compared to that induced by its higher concentrations (1 and 5 ng/ml) (Fig. 1). The most potent concentration of proliferation was 0.1 ng/ml in both groups. PBMCs of atopic dogs tended to be lesser proliferative to SEC$_{canine}$ than those of the healthy controls, with a significant difference at 0.01 ng/ml of SEC$_{canine}$ concentration ($P < 0.05$). However, there was no statistically significant difference

between the two groups treated with higher concentration of SEC_{canine}.

2. Expression of cytokine mRNA after treatment with SEC_{canine}

The mRNA expression levels of Th1 (IFN-γ, TNF- α), Th2 (IL-4, IL-5 and IL-13), and regulatory T cell (IL-10) cytokines in the PBMCs stimulated with SEC_{canine} for 24 hours were compared between atopic and healthy dogs (Fig. 2). After stimulation with SEC_{canine}, the expression levels of IFN-γ mRNA in PBMC from atopic dogs were significantly higher than those in healthy dogs ($P < 0.05$), although increased expression of SEC_{canine}-induced IFN-γ mRNA was seen in both atopic and healthy dogs. The expression levels of IL-4 and IL-13 mRNAs tended to be increased, without a significant difference between atopic (4.9 and 3.6 fold) and healthy dogs (1.7 and 1.6 fold). On the other hand, the expression levels of IL-5, IL-10, and TNF-α were not induced by SEC_{canine}.

Discussion

Canine atopic dermatitis is a common skin disease affecting approximately 10 % of the canine population (Hillier and Griffin, 2001). Although the pathogenesis of AD remains unclear, an imbalance between Th1 and Th2 cytokine response characterized by high IL-4 mRNA expression at lesion sites (Nuttall et al., 2002) and low IFN-γ mRNA expression in peripheral blood mononuclear cells (Hayashiya et al., 2002) has been reported in dogs.

The present study demonstrated for the first time about the proliferation and production of cytokines in response to SEC_{canine} in peripheral blood mononuclear cells (PBMC) from atopic and healthy dogs. In order to investigate the role of SEC_{canine} in modulating the immune response in canine AD, recombinant SEC_{canine} protein (approximately 27 kDa) were purified from a clinical isolate of *S. pseudintermedius*.

In this study, proliferation of PBMC by SEC_{canine} have been induced in both atopic and control group (Fig. 1). In dogs, stimulation of SEA and SEB to canine peripheral blood mononuclear cells *in vitro* has shown to induce blastogenesis of T cells (Hendricks, Schuberth et al. 2002). This study also revealed that the proliferative response to SEC_{canine} was significantly increased compared with that of unstimulated controls in both atopic and healthy dogs.

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Interestingly, the concentration of SEC_{canine} as low as 0.01 ng/ml induced potent T cell proliferation and lower concentration of SEC_{canine} tend to be more potent than higher concentration. This may indicate SAg-mediated death of some cell populations by higher toxin concentrations as reported previously. MHC class II expressing cells, including T cells, B cells and monocytes have been reported to be susceptible to superantigen-dependent cellular cytotoxicity (SDCC) (Hedlund, Dohlsten et al. 1990, Wallgren, Festin et al. 1993, Hendricks, Schuberth et al. 2002). Since canine T cells constitutively express MHC class II molecules (Doveren, Buurman et al. 1985), it is possible that SDCC may be responsible for the reduced proliferative response to higher concentration of SEC_{canine} in the present study.

Despite a similar degree of T cell proliferation induced by mitogen PHA, the present study showed different proliferative responses of PBMC to SEC_{canine} in atopic and healthy dogs. PBMCs of atopic dogs appear to be less responsive to SEC_{canine} than those of the healthy controls, with a significant difference at 0.01 ng/ml of SEC_{canine} concentration ($P < 0.05$). A possible explanation is that repeated exposure to SE may induce non-responsiveness in AD T cells by reprogramming immunocytes including T cells to enter an anergic state. Consistent with a previous report, less proliferative responses of PBMCs in atopic dogs may be secondary to T cell death by apoptosis (Yoshino, Asada et al. 2000). At higher concentration of SEC_{canine}, however, no statistically significant

difference was observed between PBMC proliferation of atopic and healthy dogs. The reason for this result may be related to the variability of clinical severity of atopic dogs studied. Similar studies in human AD reported that the reduced T cell proliferation in response to SEB is more pronounced during an exacerbation of the AD and is connected with the increased T cell death by apoptosis (König, Neuber et al. 1995, Yoshino, Asada et al. 2000, Fukushima, Hirano et al. 2006). Furthermore, many studies demonstrated that the clinical severity of AD correlated significantly with the skin colonization by superantigen-producing staphylococcal strains (Breuer, Wittmann et al. 2000, Bunikowski, Mielke et al. 2000, Mallinckrodt 2000, Hendricks, Schuberth et al. 2002, Kedzierska, Kaszuba-Zwoinska et al. 2005).

The existence of Th1/Th2 subsets in Th lymphocytes that differ in cytokine production patterns and effector functions provides a framework for understanding normal and pathological immune responses (Mosmann 1991). Th1 cell cytokines (IFN-γ, IL-2, TNF-α) promote the cell-mediated immunity, whereas Th2 cell cytokines (IL-4, IL-5, IL-13) support humoral immunity and IgE production, which is characteristic of atopic diseases (Nuttall, Knight et al. 2002). Other cytokines (e.g. IL-10) produce by regulatory T cells have 'regulatory' or inhibitory functions.

The present study showed that PBMC from atopic and healthy dogs stimulated by SEC$_{canine}$ produced increased mRNAs expression of IL-4 and IL-13, Th2-type

cytokines, and IFN-γ, a Th1 cytokine, in atopic dogs, but did not differ significantly between atopic and healthy dogs (Fig. 2). These results are not compatible with previous studies showing that the SEB-induced cytokine response resulted in significantly reduced IFN-γ, a Th1-type cytokine, mRNA production between atopic and non-atopic groups (Campbell and Kemp, 1997, Yoshikawa, 1999, Smart and Kemp, 2002). With regard to IFN-γ, a Th1-type cytokine, PBMC from atopic and healthy dogs stimulated by SEC_{canine} demonstrated increased levels of IFN-γ mRNA compared to those from unstimulated groups. Interestingly, after stimulation with SEC_{canine}, the expression levels of IFN-γ mRNA in PBMC from atopic dogs were significantly higher than those in healthy dogs ($P < 0.05$). In accordance with a previous report that showed increased IL-4 mRNA expression induced by SEB in human (Yoshino, Asada et al. 2000), the result revealed that not only increased level of IL-4 mRNA but also IL-13 mRNA stimulated by SEC_{canine}. IL-4 causes B cells to change antibody synthesis from IgG to IgE (class-switching). IL-13 is a critical mediator of allergic inflammation (Wills-Karp and Chiaramonte 2003).

Importantly, this study demonstrate a significantly increased Th1 cytokine (IFN-γ) response to SEC_{canine} in atopic dogs with an increased Th2 cytokines (IL-4 and IL-13) response to the same antigen. According to the results, these findings indicate an up-regulation of both Th1 and Th2 type responses induced by SEC_{canine} rather than a down-regulation of Th1 responses as has been suggested to

occur in the development of atopic disease in human. The cytokine transcription pattern in the atopic skin lesion is generally known to be changed over time with a biphasic response in atopic dogs. More specifically, Th2 cytokines may play a more important role in the acute phase, whereas Th1 cytokines may be more relevant in the chronic phase (Marsella, Olivry et al. 2006). Recent experiments have established that expression of the Th1 cytokine IFN-γ predominates in chronic skin lesions of AD, suggesting that Th2 skewing in AD is not associated with impaired expression of Th1 cytokines (Nuttall, Knight et al. 2002). In addition, a mixed cytokine transcription profile is seen in which Th1 cytokines such as IL-2, IFN-γ, and TNF-α as well as IL-4 are overexpressed in atopic skin lesion (Nuttall, Knight et al. 2002). Chronic lesions in human atopic dermatitis also exhibit a mixed pattern of cytokines, including IL-4, IL-13, IL-5, IL-2, IFN-γ, and IL-12, which could be a response to self-trauma and microbial colonization, or involve selective recruitment of allergen specific Th1 cells in late phase inflammatory responses (Werfel, Morita et al. 1996, Nuttall, Knight et al. 2002). The production of IFN-γ is thought to be related to the stage of evolution of the skin lesions (Hayashiya, Tani et al. 2002). Because the frequency of isolated superantigen from staphylococcal skin infection is thought to be correlated with atopic disease and staphylococcal colonization (Fazakerley, Nuttall et al. 2009, Eui-Hwa Nam 2013), this finding may show that SEC_{canine} leads to high levels of Th1 cytokines and thereby contribute to chronic lesions in atopic dogs.

In agreement with previous investigations about SEB in human (Yoshino, Asada et al. 2000), the production of other cytokines (IL-5, IL-10, and TNF-α) in response to SEC_{canine} was not much changed between atopic and normal dogs.

In conclusion, this study demonstrates for the first time that SEC_{canine} has a potency to proliferate T lymphocytes and to induce IFN-γ in canine PBMC. In particular, findings of responsiveness to SEC_{canine} in atopic dogs suggest that SEC_{canine} may play a key role in immune response to *S. pseudintermedius* infection, representing a potential factor for the aggravation of atopic dermatitis by reducing proliferation in T cells and the induction of a Th1 response and chronification of AD.

Table 1. Characteristics of the atopic dogs and healthy dogs.

Patient number	Group	Age (years)	Sex	Breeds
1	Atopic group	9.5	F	Miniature Schnauzer
2	Atopic group	4	M	Beagle
3	Atopic group	8	FS	Shih Tzu
4	Atopic group	1.5	MC	Cavalier King Charles Spaniel
5	Atopic group	10	MC	American Cocker Spaniel
6	Atopic group	3	MC	Bull dog
7	Atopic group	11	FS	Shih Tzu
Average age		5.8		
8	Normal group	1	M	Beagle
9	Normal group	1	M	Beagle
10	Normal group	3.5	M	Beagle
11	Normal group	1	F	Beagle

12	Normal group	3.5	F	Beagle
13	Normal group	4	F	Beagle
Average age		2.3		

M; male, MC; castrated male, F; female, FS; spayed female

Table 2. Sequences of primers used for the real-time PCR

Target Gene	Primer	Sequence (5'-3')	Nucleotide position
GAPDH	Forward	CATTGCCCTCAATGACCACT	894-998
	Reverse	TCCTTGGAGGCCATGTAGAC	
IL-4	Forward	CCAAAGAACACAAGCGATAAGGAA	98-222
	Reverse	GTTTGCCATGCTGCTGAGGTT	
IL-5	Forward	CCATGAATAGACTGGTGGCAGA	102-197
	Reverse	TTTCAGGAGTAGGAATCATCAGGT	
IL-10	Forward	ACCACGACCCAGACATCAAGA	303-461
	Reverse	CCTGGAGCTTACTAAATGCGCT	
IL-13	Forward	CTGGTCAACATCACCCAGAATCA	157-318
	Reverse	GCACAGTGCTTTCAGCATCCTC	
IFN-γ	Forward	GCGCAAGGCGATAAATGAAC	406-556
	Reverse	CTGACTCCTTTTCCGCTTCCT	
TNF-α	Forward	GAGCCGACGTGCCAATG	97-175
	Reverse	CAACCCATCTGACGGCACTA	

Table 3. Effect of SEC$_{canine}$ (0.01 - 5 ng/ml) on the proliferation of PBMC from atopic and healthy dogs using a modified water soluble tetrazolium salt (WST-1) assay after 72 hour incubation.

Patient number	PHA	SEC$_{canine}$ 0.01 ng/ml	SEC$_{canine}$ 0.1 ng/ml	SEC$_{canine}$ 1 ng/ml	SEC$_{canine}$ 5 ng/ml
1	50.27	-3.11	10.99	-6.59	0.46
2	13.77	7.05	22.3	8.85	20.98
3	41.41	13.74	27.67	-9.04	-18.63
4	44.31	-3.7	27.98	11.66	-15.5
5	39.07	57.63	58.56	30.75	24.36
6	29.84	4.8	20.09	10.92	7.13
7	3.13	2.05	9.04	11.45	7.11
8	35.84	36.55	38.84	45.42	8.17
9	53.43	55.74	73.01	44.48	22.28
10	12.08	20.18	41.13	-1.33	-33.81
11	24.85	22.38	61.77	30.81	-20.06
12	44.02	13.01	15.43	40.7	28.29

| 13 | 7.89 | 59.3 | 29.41 | -0.78 | 4.58 |
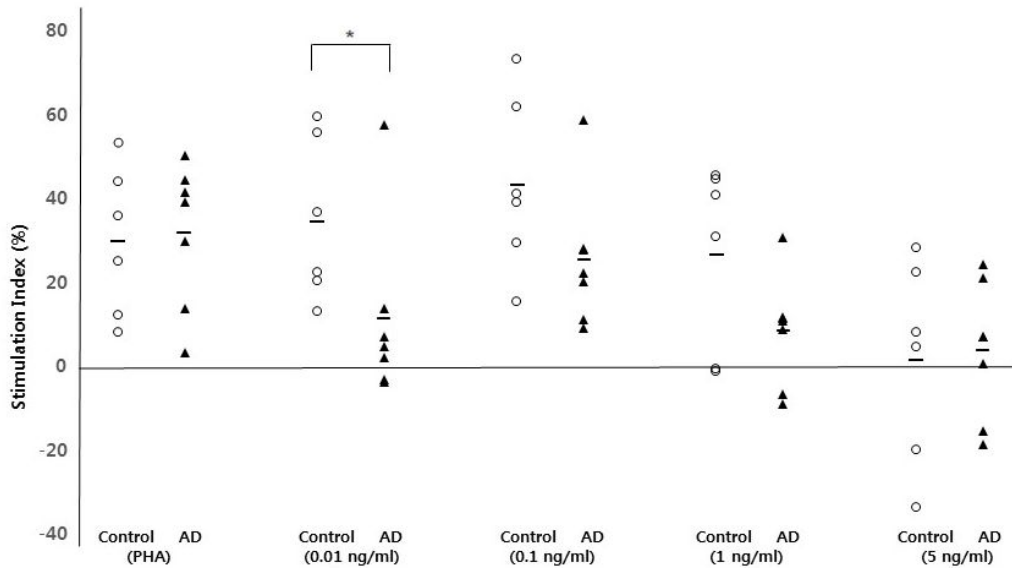
Figure 1. Effect of SEC$_{canine}$ (0.01 - 5 ng/ml) on the proliferation of PBMC from atopic and healthy dogs using a modified water soluble tetrazolium salt (WST-1) assay after 72 hour incubation. Phytohaemagglutinin (PHA) treated was used as

positive control. The stimulation index (SI) means the formula (S – C) / C x 100, with *S* as the absorbance of stimulated cells and *C* as the absorbance of control unstimulated cells. **-** Represents the median of SI.
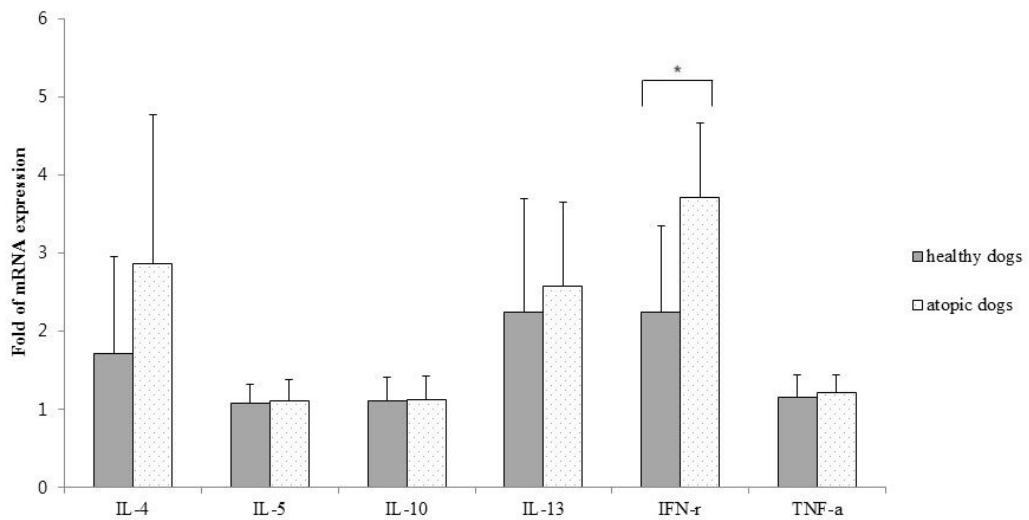
Figure 2. Relative mRNA expression levels of Th1 (TNF- α, IFN- γ), Th2 (IL-4, IL-5, IL-13), and regulatory T (IL-10) cell cytokines in PBMC from atopic (AD) and healthy dogs (control) following SEC$_{canine}$ stimulation for 24 hours. The fold of mRNA expression represents relative differences to the internal control gene

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(GAPDH) expression by subtracting Ct values of the internal control gene from Ct values of the target (ΔCt).

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Staphylococcus pseudintermedius 장독소 C형 (SEC~~canine~~)이 아토피 개의 말초혈액 단핵세포에 미치는 영향

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박 정 훈

개 아토피 피부염은 특정환경원성 항원에 의해 염증 및 소양 감을 나타내는 유전성 알러지성 피부염이며 초항원으로 작용하 는 외독소를 분비하는 *Staphylococcus pseudintermedius*의 집

락화와 관련이 있는 것으로 알려져 있다. *S. pseudintermedius* 감염에 있어서 SEC_{canine}의 높은 검출 빈도에도 불구하고 아직까지 SEC_{canine}의 중요성에 대해서 많은 부분 알려져 있지 않다. 본 연구는 SEC_{canine}가 아토피 개의 말초혈액 단핵세포에 미치는 영향을 증식반응 검사와 사이토카인의 발현을 통해 알아보고자 수행되었다. 7 마리의 아토피 개와 6 마리의 건강한 개로부터 분리한 말초혈액 단핵세포를 SEC_{canine}로 자극한 뒤 WST-1을 이용하여 증식반응을 확인하였고 real-time PCR 분석을 통하여 IL-4, IL-5, IL-10, IL-13, IFN-γ, TNF-α의 mRNA 발현을 정량 하였다. 증식반응 측정 결과 아토피군과 정상군 모두에서 낮은 농도의 SEC_{canine} 자극이 더 높은 증식반응 경향을 나타내는 것이 관찰되었고 아토피군이 정상군에 비해 더 낮은 증식반응을 나타냈다. 말초혈액 단핵세포의 사이토카인 발현량을 측정한 결과 아토피군이 정상군에 비해 IFN-γ의 발현량이 유의적으로 높게 관찰되었다. 상기 결과들은 SEC_{canine}가 아토피 개의 말초혈액 단핵세포에서 정상군에 비해 낮은 증식반응을 일으

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키고 T_H1 도움 T 세포 의 주요 사이토카인인 IFN$-\gamma$ 의 발현을 증가시킨다는 것을 나타낸다. 따라서 SEC$_{canine}$를 분비하는 *S. pseudintermedius* 감염은 T 세포의 증식반응을 감소시키고 병변의 T_H1 도움 T 세포 우세화를 유도함으로써 병변의 악화 및 만성화를 촉진시키는 요인으로 작용할 수 있을 것으로 생각된다.