



Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm



Short communication

House dust mite major allergen Der f 1 enhances proinflammatory cytokine and chemokine gene expression in a cell line of canine epidermal keratinocytes

Shingo Maeda^a, Sadatoshi Maeda^{b,*}, Sanae Shibata^c, Naoki Chimura^c, Tsuneo Fukata^c

^a Department of Veterinary Medicine, Faculty of Applied Biological Sciences, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan

^b Department of Veterinary Internal Medicine, Faculty of Applied Biological Sciences, Gifu University, Gifu, Japan

^c The United Graduate School of Veterinary Sciences, Gifu University, Gifu, Japan

ARTICLE INFO

Article history:

Received 8 January 2009

Received in revised form 24 March 2009

Accepted 14 April 2009

Keywords:

Dog

House dust mite

Der f 1

Inflammatory cytokine

Atopic dermatitis

Keratinocyte

ABSTRACT

House dust mite (HDM) allergens are the most common allergens involved in the induction of IgE-mediated hypersensitivity. Recently, epicutaneous sensitization with HDM allergens has been emphasized in the development of atopic dermatitis (AD); however, direct stimulation of canine keratinocytes by mite allergens has not been well investigated. In the present study, we investigated the effects of Der f 1, a major allergen of *Dermatophagoides farinae*, on cytokine and chemokine gene expression in a canine keratinocyte cell line, CPEK. CPEK constitutively expressed mRNA for TNF- α , IL-12p35, IL-18, GM-CSF, TGF- β , IL-8/CXCL8, TARC/CCL17, CTACK/CCL27 and MEC/CCL28. Of all the cytokines and chemokines investigated in CPEK, transcription levels of GM-CSF, IL-8/CXCL8 and TNF- α mRNA were significantly enhanced by stimulation with Der f 1. The present results suggest that Der f 1 can directly augment inflammatory cytokine and chemokine production from keratinocytes, and may initiate allergic inflammation independently of Type-I hypersensitivity.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Canine atopic dermatitis (AD) is 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). Type-I hypersensitivity is mediated by allergens, allergen-specific IgE and mast cells. Of the various aeroallergens, house dust mite (HDM), *Dermatophagoides farinae* and *D. pteronyssinus*, allergens are the most common in temperate climates, and are known to be associated with the immunopathogenesis of canine AD (Lian and Halliwell, 1998; Masuda et al., 2000) as well as a human counterpart (Yasueda et al., 1989).

Historically, respiratory sensitization has been thought to be important to develop AD; however, recent studies have revealed that epicutaneous sensitization played a crucial role in the development of AD. In a canine model of AD, Pucheu-Haston et al. demonstrated that HDM allergens can penetrate the epidermis, inducing elevations in mite-specific IgE and pruritic dermatitis (Pucheu-Haston et al., 2008). In addition, a previous study showed that atopy patch testing with HDM enhanced mRNA transcription of IL-6, IL-12p35, IL-13, IL-18, IFN- γ and thymus and activation regulated chemokine (TARC/CCL17) in lesional skin of high-IgE beagles (Marsella et al., 2006). It has been well known that these cytokine and chemokine mRNA levels are enhanced in AD skin lesions (Leung, 2000). Lymphocytes, mast cells, macrophages, eosinophils, and Langerhans cells are considered the cell source of these cytokines and chemokines. Among these cells, it has been well recognized that lymphocytes can produce various

* Corresponding author. Tel.: +81 58 293 2896; fax: +81 58 293 2896.
E-mail address: sadat@gifu-u.ac.jp (S. Maeda).

cytokines; however, recent studies have revealed that basal keratinocytes have the potential to produce cytokines (Gröne, 2002) and chemokines (Kagami et al., 2006; Vestergaard et al., 2000). These results suggest that allergens infiltrating the epidermis may initiate allergic inflammation by lesional production of cytokines and chemokines independently of Type-I hypersensitivity; however, no study has reported the activation of canine keratinocytes by HDM allergens. In this study, we investigated the effects of HDM major allergen Der f 1 on cytokine and chemokine gene expression in a canine epidermal keratinocyte cell line, CPEK.

2. Materials and methods

2.1. Materials

Opti-MEM I reduced-serum medium and TrypLE Express were purchased from Invitrogen Corp. (Carlsbad, CA). CnT-09 was supplied by CELLnTEC Advanced Cell Systems (Bern, Switzerland). Dulbecco's phosphate-buffered saline (DPBS) and antibiotic antimycotic solution were purchased from Sigma–Aldrich (St. Louis, MO). Purified natural Der f 1 was obtained from Seikagaku Biobusiness Corp. (Tokyo, Japan).

2.2. Cell culture

A cell line derived from the epidermis of an adult beagle, CPEK (CELLnTEC), was cultured in 75 cm² flasks (Nunc Inc., Roskilde, Denmark) in CnT-09 with 1% antibiotic antimycotic solution until approximately 80% confluence at 37 °C under 5% CO₂. The cultured cells were trypsinized by treatment with 2 ml TrypLE Express and incubated for 7–10 min at 37 °C. When approximately 90% of the cells had been detached, the cell suspension was transferred to a sterile 15 ml centrifuge tube (Nunc Inc.) with DPBS and centrifuged at 180 × g for 5 min at room temperature. The cell pellet was gently re-suspended in CnT-09 and cultured in Nunclon Multidish (24-wells, Nunc Inc.) until 100% confluence. In this study, 5th- to 10th-passage CPEK was used. When cultured CPEK had reached 100% confluence in CnT-09, the cells were washed two times with DPBS and cultured in serum-free Opti-MEM I reduced-serum medium 24 h before stimulation with Der f 1.

2.3. Total RNA extraction from CPEK stimulated with Der f 1

Der f 1 solution (100 µg/ml) was prepared by dissolving 50 µg of lyophilized Der f 1 in 500 µl of DPBS. CPEK was incubated in 24-well plate with 0.9 ml fresh Opti-MEM containing 0.1 ml of DPBS (control) or 0.1 ml of Der f 1 solution (stimulated samples). After the indicated incubation period (12 h, 24 h and 48 h), total RNA was extracted with a commercially available kit (RNeasy Mini Kit; Qiagen Inc., Valencia, CA) according to the manufacturer's instructions. Genomic DNA was removed from the samples with a TURBO DNA-free Kit (Applied Biosystems, Foster City, CA), and then stored at –80 °C until further analysis. At least three independent experiments were performed.

2.4. Detection of constitutive transcription of cytokine and chemokine mRNA in CPEK

Reverse-transcription polymerase-chain reaction (RT-PCR) was used to detect the constitutive transcription of cytokine and chemokine mRNA in CPEK. Total RNA was reverse-transcribed to cDNA using a commercially available kit (ReverTra Ace-α-; Toyobo, Osaka, Japan) according to the manufacturer's instructions. RT-PCR was performed on a thermal cycler (Takara Bio Inc., Shiga, Japan) using TaKaRa Ex Taq (Takara Bio Inc.). Sequences of the primer pairs used for PCR are shown in Table 1. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA was used as an internal control. The PCR amplifications consisted of denaturation (94 °C for 2 min), and 40 cycles of denaturation (94 °C for 1 min), annealing (60 °C for 1 min) and extension (72 °C for 1 min), followed by a final extension (72 °C for 8 min). The PCR products were electrophoresed through a 3% agarose gel and stained with ethidium bromide for visualization.

2.5. Quantification of mRNA transcription for GM-CSF, TNF-α, TARC/CCL17 and IL-8/CXCL8 in CPEK stimulated with Der f 1

Transcription analysis of GM-CSF, TNF-α, TARC/CCL17 and IL-8/CXCL8 mRNA in CPEK treated with or without Der f 1 was quantified by one-step real-time PCR (Thermal Cycler Dice Real Time System; Takara Bio Inc.) using a commercial kit (One-Step SYBR PrimeScript RT-PCR Kit II; Takara Bio Inc.) according to the manufacturer's instructions. Transcription of GAPDH mRNA was used as an internal control. Sequences of the primer pairs used for quantitative PCR are shown in Table 1. One-step real-time PCR consisted of reverse-transcription (42 °C for 5 min and 95 °C for 10 s), and 40 cycles of PCR reaction (95 °C for 5 s and 60 °C for 30 s), followed by dissociation (95 °C for 15 s, 60 °C for 30 s and 95 °C for 15 s). mRNA transcription was quantified using the comparative cycle threshold (Ct) method, by which the relative transcription of the target gene (cytokines and chemokines) is reported as the *n*-fold difference relative to that of the calibrator gene (GAPDH). For this purpose, Ct values of the calibrator gene were subtracted from Ct values of the target cytokine or chemokine (ΔCt). All samples were examined in duplicate and the mean value of ΔCt was calculated. The amount of cytokine and chemokine mRNA was calculated by 2^{–ΔΔCt}, evaluating the samples as the *n*-fold difference relative to the calibrator gene. Finally, mean relative transcription was described by the ratio of the stimulated samples to unstimulated samples with standard deviation (S.D.) of three independent experiments.

2.6. Statistical analysis

The data were statistically analyzed using Dunnett's test to compare cytokine and chemokine mRNA transcription levels among the different culture periods. All analyses were performed using a statistical software

Table 1
Sequences of oligonucleotide primers used for RT-PCR and quantitative real-time PCR.

Primer set		Primer sequence (5'–3')	Position of the primers	Genbank accession number
TNF- α	Forward	CCCAAGTGACAAGCCAGTAGCTC	249–394	NM_001003244
	Reverse	ACAACCCATCTGACGGCACTATC		
IL-1 β	Forward	ACCCGAACCTACCAAGTGAAATG	12–121	NM_001037971
	Reverse	GGTTCAGGTCTTGGCAGCAG		
IL-4	Forward	TCTGCTTACTAGCACTACCAGCAC	98–222	NM_001003159
	Reverse	GACAGTCAGCTCCATGCACGA		
IL-5	Forward	CCATGAATAGACTGGTGGCAGA	102–197	NM_001006950
	Reverse	TTTCAGGAGTAGGAATCATCAGGT		
IL-6	Forward	TCTGTGCATAGTAGTACCAAGATCC	466–590	NM_001003301
	Reverse	TCCTGCGACTGCAAGATAGCC		
IL-12p35	Forward	AGAGTTGCCTGGCTTCCAGAGA	290–397	NM_001003293
	Reverse	CCTCATAGATGCTGCTAAGGCACA		
IL-13	Forward	CTGGTCAACATCACCCAGAATCA	157–318	NM_001003384
	Reverse	GCACAGTGCTTTCAGCATCTCTC		
IL-18	Forward	ACTTTGGCAAGCTTGAACCTAAAC	110–291	NM_001003169
	Reverse	TGCCAGACCTCTAGTGAGGTATC		
IFN- γ	Forward	CTTGGCAAGTTCTTAATAGCAGCA	406–556	NM_001003174
	Reverse	TCCTTAGGTTGGATCTTGGTGAGAG		
GM-CSF	Forward	GATGGCCAATCACTATAAGCAGCA	294–433	NM_001003245
	Reverse	TGACTGGTTTCCAGCAGTCAAAG		
TGF- β	Forward	GGAGCAGCATGTGGAGCTGTA	549–673	NM_001003309
	Reverse	GCCTCAGACTCCAGTGACATC		
IL-8/CXCL8	Forward	CTTCCAAGCTGGCTGTGCTC	11–183	NM_001003200
	Reverse	TGGGCCACTGTCAATCACTCTC		
TARC/CCL17	Forward	GGCTGACAAGGTGGTACAAGACTTC	137–218	NM_001003051
	Reverse	CAGATGGACTTGCTTGGACAG		
CTACK/CCL27	Forward	AGCCACTCTCGAACAAGCTACTGA	119–226	NM_001003968
	Reverse	CACTGCGTCGAGACAGGTGAA		
MEC/CCL28	Forward	CAGACAGGACTCACTCTCGCTCTC	7–113	NM_001005257
	Reverse	TGTGAAACCTCAGTGCAACAGCTA		
GAPDH	Forward	CATTGCCCTCAATGACCACT	894–998	NM_001003142
	Reverse	TCCTTGAGGCCATGTAGAC		

package (JMP version 5.1.2; SAS Institute, Cary, NC). Statistical significance was defined as $P < 0.05$.

3. Results and discussion

Among the cytokines and chemokines investigated in this study, CPEK constitutively expressed TNF- α , IL-12p35, IL-18, GM-CSF, TGF- β , IL-8/CXCL8, TARC/CCL17, CTACK/CCL27 and MEC/CCL28 (data not shown). Basal keratinocytes in other species are known to be a major cell source of cytokines (Gröne, 2002) and chemokines (Kagami et al., 2005; Vestergaard et al., 2001). In this study, the results of RT-PCR analysis also confirmed the constitutive transcription of cytokine and chemokine mRNA in CPEK. Among the cytokines and chemokines expressed in CPEK, TARC/CCL17 is known to be a very important chemokine in the pathogenesis of AD (Imai et al., 1999; Imai et al., 1996). Because of constitutive TARC/CCL17 secretion, HaCaT, a human keratinocyte cell line, has been used as a keratinocyte model of AD *in vitro* (Vestergaard et al., 2000). Since all cytokines and chemokines constitutively expressed in CPEK are also produced by HaCaT (Bachmeier

et al., 2002; Kagami et al., 2006; Park et al., 2007; Vestergaard et al., 2000; Yoshizumi et al., 2008), CPEK appears to be similar to HaCaT in cytokine and chemokine profiles. Previous study also indicated that CPEK showed classical cobble appearance of undifferentiated keratinocytes with expression of markers for basal keratinocyte such as cytokeratin 14, α_6 integrin and proliferating cell nuclear antigen (Shibata et al., 2008); therefore, CPEK is thought to be suitable as an *in vitro* experimental tool for studying the immunopathogenesis of canine AD.

In this study, the transcription of GAPDH mRNA was used as an internal control to correct the transcription rate of cytokine and chemokine mRNA after Der f 1 stimulation; however, there is a possibility that Der f 1 treatment changed the transcription level of GAPDH mRNA in CPEK. To evaluate GAPDH as an internal control, Ct values of GAPDH were compared between stimulated and unstimulated CPEK. Statistical analysis demonstrated that there was no difference in Ct values between stimulated and unstimulated samples (data not shown), which indicated that GAPDH could be used as an appropriate internal control in this experiment.

Table 2

Constitutive transcription levels of the cytokine and chemokine mRNA in CPEK at different culture time.

	0 h	12 h	24 h	48 h
TARC/CCL17	$1.5 \pm 0.8 \times 10^{-4}$	$7.8 \pm 3.7 \times 10^{-4}$	$6.8 \pm 3.0 \times 10^{-4}$	$12.5 \pm 8.1 \times 10^{-4}$
GM-CSF	$3.1 \pm 0.7 \times 10^{-5}$	$0.3 \pm 0.1 \times 10^{-5}$	$0.3 \pm 0.2 \times 10^{-5}$	$1.3 \pm 0.9 \times 10^{-5}$
IL-8/CXCL8	$3.2 \pm 0.6 \times 10^{-2}$	$1.6 \pm 0.3 \times 10^{-2}$	$2.2 \pm 0.6 \times 10^{-2}$	$5.2 \pm 3.2 \times 10^{-2}$
TNF- α	$4.8 \pm 1.9 \times 10^{-5}$	$3.0 \pm 2.4 \times 10^{-5}$	$2.3 \pm 3.2 \times 10^{-5}$	$3.9 \pm 5.1 \times 10^{-5}$

The amount of cytokine and chemokine mRNA was calculated by $2^{-\Delta\Delta C_t}$, evaluating the samples as the *n*-fold difference relative to the calibrator gene.

In the pilot study, we investigated whether Der f 1 would enhance mRNA transcription for cytokines and chemokines expressed constitutively in CPEK. Of all the cytokines and chemokines investigated, transcription of GM-CSF, TNF- α , TARC/CCL17 and IL-8/CXCL8 mRNA was found to be enhanced after stimulation with Der f 1 (data not shown); therefore, later experiments focused on transcription kinetics of these cytokines and chemokines. Constitutive transcription levels of these cytokine and chemokine mRNA in CPEK at different culture time are shown in Table 2. Except for TARC/CCL17, the levels were not significantly changed during the culture period.

The present results clearly indicated that Der f 1 significantly enhanced mRNA transcription of IL-8/CXCL8, GM-CSF and TNF- α (Fig. 1). Notably, mRNA transcription of IL-8/CXCL8 and TNF- α increased approximately 30-fold after treatment (Fig. 1c and d). IL-8/CXCL8 is a functional ligand for CXC chemokine receptor 1 (CXCR1) and CXCR2, and attracts neutrophils. Since neutrophil infiltration is not a characteristic finding in atopic lesions, augmentation of IL-8/CXCL8 mRNA transcription enhanced by Der f 1 might not be directly associated with the immunopathogenesis of AD but may represent an aspect of host defense to environmental stimuli. TNF- α is a proinflammatory cytokine that stimulates vascular endothelial cells to express new adhesion molecules and induces various cells

to secrete chemokines. A previous study reported that TNF- α was an important soluble factor to produce TARC/CCL17 in HaCaT (Vestergaard et al., 2000). In this study, however, the transcription level of TARC/CCL17 mRNA was not significantly enhanced by stimulation with Der f 1 (Fig. 1a), although keratinocytes appeared to produce TNF- α . This may have been due to the relatively short culture period, which is not enough for TNF- α accumulation produced from CPEK itself. In the present study, active transcription of TNF- α was found at 48 h; therefore, transcription of TARC/CCL17 mRNA would be increased during a culture period beyond 48 h. The transcription level of GM-CSF mRNA was also significantly enhanced by stimulation with Der f 1 (Fig. 1b). In lesional skin of human AD, keratinocytes have been shown to overproduce GM-CSF (Pastore et al., 1997). In the epidermis, GM-CSF mediates the differentiation of Langerhans cells into mature dendritic cells (Heufner et al., 1988; Witmer-Pack et al., 1987) and stimulates keratinocyte proliferation *in vitro* (Hancock et al., 1988). Moreover, Tada et al. reported that GM-CSF produced by keratinocytes inhibited IL-12, a promoter cytokine of the development of Th1 cells, production from Langerhans cells in mice (Tada et al., 2000); therefore, up-regulation of GM-CSF induced by Der f 1 may enhance allergen presentation by dendritic cells, acanthosis and development of Th2 cells, deteriorating clinical signs of AD.

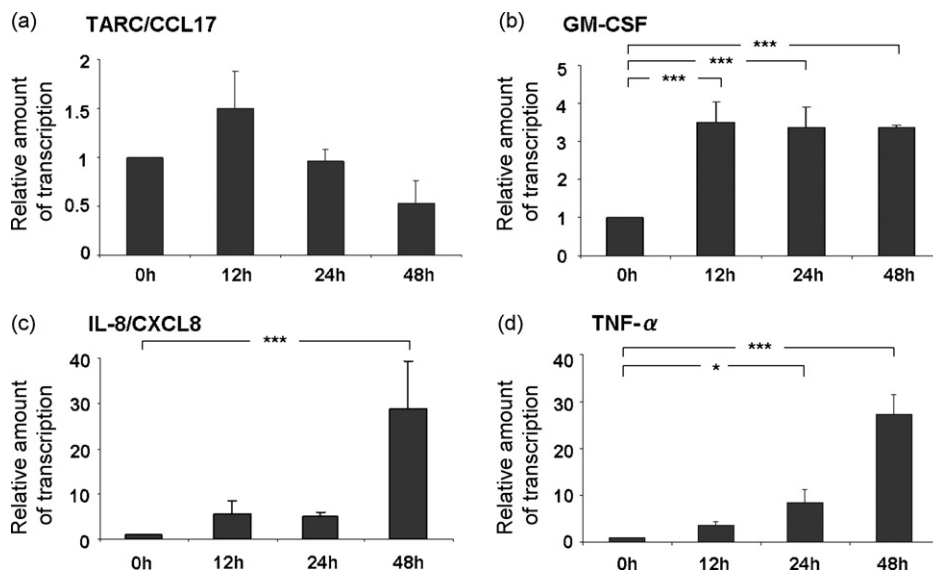


Fig. 1. Transcription levels of TARC/CCL17 (a), GM-CSF (b), IL-8/CXCL8 (c) and TNF- α (d) mRNA in CPEK stimulated with Der f 1. CPEK was incubated with medium alone or Der f 1 (10 μ g/ml) for the indicated period. Mean relative amounts of transcription are described by the ratios of stimulated to unstimulated samples. Error bars represent S.D. of three independent experiments. **P* < 0.05, ****P* < 0.001.

Although several studies have reported that Der f 1 induces IL-8/CXCL8 and GM-CSF production from human keratinocytes (Kato et al., 2005; Ogawa et al., 2008) in canine keratinocytes, the present study is the first to demonstrate the gene expression of IL-8/CXCL8, GM-CSF and TNF- α enhanced by Der f 1.

Although the mechanism of keratinocyte activation mediated by Der f 1 was not investigated in this study, protease-activated receptor-2 (PAR-2) is most likely involved. Asokanathan et al. demonstrated that the cysteine protease activity of Der p 1 stimulated the production of proinflammatory cytokines from respiratory epithelial cells through the activation of PAR-2 (Asokanathan et al., 2002). A recent study indicated that both Der p 1 and Der f 1 have cysteine protease (Takai et al., 2005). In this study, therefore, it is conceivable that the up-regulation of cytokines and chemokines mRNA transcription may be resulted from the interaction between Der f 1 and PAR-2. To prove this hypothesis, further experiments using PAR-2 antagonist or small interfering RNAs to knock down endogenous PAR-2 expression will be necessary.

In conclusion, in CPEK, it was found that Der f 1 enhanced TNF- α , GM-CSF and IL-8/CXCL8 mRNA transcription but not TARC/CCL17. The present results indicate that keratinocytes have the potential to produce various proinflammatory cytokines and chemokines upon interaction with HDM allergens, providing new insights into the immunopathogenesis of canine AD.

Acknowledgements

This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of the Japanese Government.

References

- Asokanathan, N., Graham, P.T., Stewart, D.J., Bakker, A.J., Eidne, K.A., Thompson, P.J., Stewart, G.A., 2002. House dust mite allergens induce proinflammatory cytokines from respiratory epithelial cells: the cysteine protease allergen, Der p 1, activates protease-activated receptor (PAR)-2 and inactivates PAR-1. *J. Immunol.* 169, 4572–4578.
- Bachmeier, B.E., Ruoss, I., Hagedorn, H.G., Nerlich, A.G., 2002. Molecular analysis of TGF- β and their receptors in human keratinocyte cell lines of different biological behaviour. *Int. J. Mol. Med.* 10, 371–376.
- Gröne, A., 2002. Keratinocytes and cytokines. *Vet. Immunol. Immunopathol.* 88, 1–12.
- Halliwell, R., 2006. Revised nomenclature for veterinary allergy. *Vet. Immunol. Immunopathol.* 114, 207–208.
- Hancock, G.E., Kaplan, G., Cohn, Z.A., 1988. Keratinocyte growth regulation by the products of immune cells. *J. Exp. Med.* 168, 1395–1402.
- Heufler, C., Koch, F., Schuler, G., 1988. Granulocyte/macrophage colony-stimulating factor and interleukin 1 mediate the maturation of murine epidermal Langerhans cells into potent immunostimulatory dendritic cells. *J. Exp. Med.* 167, 700–705.
- Imai, T., Nagira, M., Takagi, S., Kakizaki, M., Nishimura, M., Wang, J., Gray, P.W., Matsushima, K., Yoshie, O., 1999. Selective recruitment of CCR4-bearing Th2 cells toward antigen-presenting cells by the CC chemokines thymus and activation-regulated chemokine and macrophage-derived chemokine. *Int. Immunol.* 11, 81–88.
- Imai, T., Yoshida, T., Baba, M., Nishimura, M., Kakizaki, M., Yoshie, O., 1996. Molecular cloning of a novel T cell-directed CC chemokine expressed in thymus by signal sequence trap using Epstein-Barr virus vector. *J. Biol. Chem.* 271, 21514–21521.

- Kagami, S., Kakinuma, T., Saeki, H., Tsunemi, Y., Fujita, H., Sasaki, K., Nakamura, K., Takekoshi, T., Kishimoto, M., Mitsui, H., Komine, M., Asahina, A., Tamaki, K., 2005. Increased serum CCL28 levels in patients with atopic dermatitis, psoriasis vulgaris and bullous pemphigoid. *J. Invest. Dermatol.* 124, 1088–1090.
- Kagami, S., Saeki, H., Komine, M., Kakinuma, T., Nakamura, K., Tsunemi, Y., Sasaki, K., Asahina, A., Tamaki, K., 2006. CCL28 production in HaCaT cells was mediated by different signal pathways from CCL27. *Exp. Dermatol.* 15, 95–100.
- Kato, T., Takai, T., Mitsuishi, K., Okumura, K., Ogawa, H., 2005. Cystatin A inhibits IL-8 production by keratinocytes stimulated with Der p 1 and Der f 1: biochemical skin barrier against mite cysteine proteases. *J. Allergy Clin. Immunol.* 116, 169–176.
- Leung, D.Y., 2000. Atopic dermatitis: new insights and opportunities for therapeutic intervention. *J. Allergy Clin. Immunol.* 105, 860–876.
- Lian, T.M., Halliwell, R.E., 1998. Allergen-specific IgE and IgG antibodies in atopic and normal dogs. *Vet. Immunol. Immunopathol.* 66, 203–223.
- Marsella, R., Olivry, T., Maeda, S., 2006. Cellular and cytokine kinetics after epicutaneous allergen challenge (atopy patch testing) with house dust mites in high-IgE beagles. *Vet. Dermatol.* 17, 111–120.
- Masuda, K., Sakaguchi, M., Fujiwara, S., Kurata, K., Yamashita, K., Odagiri, T., Nakao, Y., Matsuki, N., Ono, K., Watari, T., Hasegawa, A., Tsujimoto, H., 2000. Positive reactions to common allergens in 42 atopic dogs in Japan. *Vet. Immunol. Immunopathol.* 73, 193–204.
- Ogawa, T., Takai, T., Kato, T., Kikuchi, Y., Niyonsaba, F., Ikeda, S., Okumura, K., Ogawa, H., 2008. Upregulation of the release of granulocyte-macrophage colony-stimulating factor from keratinocytes stimulated with cysteine protease activity of recombinant major mite allergens. Der f 1 and Der p 1. *Int. Arch. Allergy Immunol.* 146, 27–35.
- Park, H.J., Kim, H.J., Lee, J.Y., Cho, B.K., Gallo, R.L., Cho, D.H., 2007. Adrenocorticotropin hormone stimulates interleukin-18 expression in human HaCaT keratinocytes. *J. Invest. Dermatol.* 127, 1210–1216.
- Pastore, S., Fanale-Belasio, E., Albanesi, C., Chinni, L.M., Giannetti, A., Girolomoni, G., 1997. Granulocyte macrophage colony-stimulating factor is overproduced by keratinocytes in atopic dermatitis. Implications for sustained dendritic cell activation in the skin. *J. Clin. Invest.* 99, 3009–3017.
- Pucheu-Haston, C.M., Jackson, H.A., Olivry, T., Dunston, S.M., Hammerberg, B., 2008. Epicutaneous sensitization with *Dermatophagoides farinae* induces generalized allergic dermatitis and elevated mite-specific immunoglobulin E levels in a canine model of atopic dermatitis. *Clin. Exp. Allergy* 38, 667–679.
- Shibata, S., Maeda, S., Tsuchida, H., Fukata, T., 2008. Phenotypic analysis for a cell line of canine epidermal keratinocytes. *J. Vet. Med. Sci.* 70, 853–855.
- Tada, Y., Asahina, A., Nakamura, K., Tomura, M., Fujiwara, H., Tamaki, K., 2000. Granulocyte/macrophage colony-stimulating factor inhibits IL-12 production of mouse Langerhans cells. *J. Immunol.* 164, 5113–5119.
- Takai, T., Kato, T., Sakata, Y., Yasueda, H., Izuhara, K., Okumura, K., Ogawa, H., 2005. Recombinant Der p 1 and Der f 1 exhibit cysteine protease activity but no serine protease activity. *Biochem. Biophys. Res. Commun.* 328, 944–952.
- Vestergaard, C., Bang, K., Gesser, B., Yoneyama, H., Matsushima, K., Larsen, C.G., 2000. A Th2 chemokine, TARC, produced by keratinocytes may recruit CLA⁺ CCR4⁺ lymphocytes into lesional atopic dermatitis skin. *J. Invest. Dermatol.* 115, 640–646.
- Vestergaard, C., Kirstein, N., Gesser, B., Mortensen, J.T., Matsushima, K., Larsen, C.G., 2001. IL-10 augments the IFN- γ and TNF- α induced TARC production in HaCaT cells: a possible mechanism in the inflammatory reaction of atopic dermatitis. *J. Dermatol. Sci.* 26, 46–54.
- Witmer-Pack, M.D., Olivier, W., Valinsky, J., Schuler, G., Steinman, R.M., 1987. Granulocyte/macrophage colony-stimulating factor is essential for the viability and function of cultured murine epidermal Langerhans cells. *J. Exp. Med.* 166, 1484–1498.
- Yasueda, H., Mita, H., Yui, Y., Shida, T., 1989. Comparative analysis of physicochemical and immunochemical properties of the two major allergens from *Dermatophagoides pteronyssinus* and the corresponding allergens from *Dermatophagoides farinae*. *Int. Arch. Allergy Appl. Immunol.* 88, 402–407.
- Yoshizumi, M., Nakamura, T., Kato, M., Ishioka, T., Kozawa, K., Wakamatsu, K., Kimura, H., 2008. Release of cytokines/chemokines and cell death in UVB-irradiated human keratinocytes, HaCaT. *Cell Biol. Int.* 32, 1405–1411.