



Inhibitory effects of *Cinnamomum cassia* extract on atopic dermatitis-like skin lesions induced by mite antigen in NC/Nga mice

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ABSTRACT

Aim of the study: *Cinnamomum cassia* (*C. cassia*) has been traditionally used to treat allergic disease as well as dyspepsia, gastritis, and blood circulation disturbances. However, the antiallergic properties of *C. cassia* have not been fully verified using scientific tools. This study investigated the effectiveness of *C. cassia* extract (CCE) as an antiallergic agent in atopic dermatitis model and underlying mechanism.

Materials and methods: The effect of CCE on mite antigen-treated NC/Nga mice was evaluated by examining skin symptom severity, levels of serum IgE, tumor necrosis factor- α (TNF- α), and histamine, skin histology, and mRNA expression of cytokines in the skin lesions. Moreover, the effect of CCE on TNF- α - and interferon- γ (IFN- γ)-induced chemokine production in human keratinocytes was investigated using ELISA.

Results: CCE treatment of NC/Nga mice reduced the dermatitis score and the levels of serum IgE, histamine, and TNF- α . Histological examination showed inhibition of the thickening of the epidermis/dermis and reduced dermal infiltration of inflammatory cells. In skin lesions, mRNA expression of IL-4, TNF- α , and thymus and activation-regulated chemokine (TARC) was inhibited by CCE treatment. The production of TARC, macrophage-derived chemokine, and RANTES from IFN- γ - and TNF- α -stimulated human keratinocytes was suppressed by CCE treatment in a dose-dependent manner.

Conclusions: CCE inhibits the development of atopic dermatitis-like skin lesions in NC/Nga mice by suppressing the T-helper 2 cell response.

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1. Introduction

Atopic dermatitis is a chronic inflammatory skin disease with an increasing prevalence in industrialized countries. It commonly presents during early infancy and childhood but also can persist into or start in adulthood (Spergel and Paller, 2003). The major clinical symptoms of atopic dermatitis are pruritic and chronic eczematous skin lesions that are distinguished by infiltration of inflammatory cells consisting of T lymphocytes, monocytes/macrophages, eosinophils, and mast cells (Soter, 1989; Leung and Bieber, 2003). In atopic dermatitis, skin injury due to environmental allergens, scratching, or microbial toxins activates keratinocytes to induce production of proinflammatory cytokines (Leung et al., 2004). Various chemokines and adhesion molecules

are induced by these proinflammatory cytokines, leading to the recruitment of pathogenic leukocytes to the skin (Homey et al., 2006). T-helper 2 (Th2)-type T cells, which produce IL-4, IL-5, and IL-10, play major roles in acute atopic dermatitis, and Th1-type cytokines such as interferon- γ (IFN- γ) are involved in chronic atopic dermatitis (Vestergaard et al., 1999; Leung et al., 2004). In most patients with atopic dermatitis, IgE levels in the circulation are elevated, which is attributed to the high production of IL-4, an inducer of IgE production.

The inbred NC/Nga mouse strain was established in 1957 using a Japanese mouse strain (Nishiki-Nezumi). When these mice are placed in conventional surroundings and not maintained under specific pathogen-free (SPF) conditions, clinical signs including scratching behavior appear, and IgE levels are elevated beginning at 8 weeks of age, followed by the onset of eczematous conditions and infiltration of various inflammatory cells into the skin lesions (Matsuda et al., 1997; Vestergaard et al., 1999). Thus, NC/Nga mice are very useful animal models for investigating the pathogenesis of human atopic dermatitis. Patients with atopic dermatitis are highly sensitized to house dust mite allergens. The body and feces

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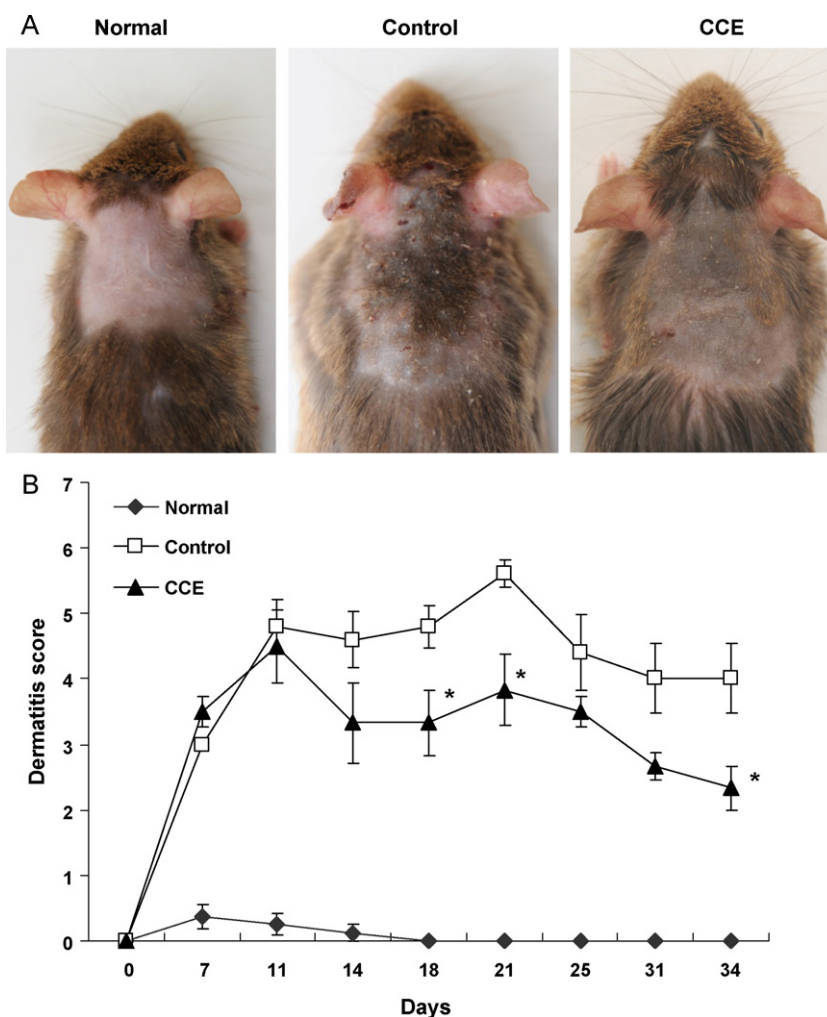


Fig. 1. Effect of CCE on the development of dermatitis in NC/Nga mice. (A) Clinical features. (B) The dermatitis score was assessed with the criteria described in Section 2. Values are expressed as means $\pm$ S.E. of 7–8 animals. * $P < 0.05$ vs. control group.

of *Dermatophagoides farinae*, a major species of house dust mites, are well-known major environmental allergens. Previously, it was reported that *D. farinae* extract (DFE) induces atopic dermatitis-like skin lesions in NC/Nga mice (Matsuoka et al., 2003; Yamamoto et al., 2007).

In general, topical steroid therapy is crucial for the treatment of atopic dermatitis, but it cannot be administrated long term due to its side-effects (Leung and Bieber, 2003). Results from several studies indicate that patients with atopic dermatitis may benefit from oriental herbal medicine therapy (Gao et al., 2005; Kim et al., 2008). *Cinnamomum caccia* Presl (*C. cassia*), also known as Chinese cinnamon, is a small evergreen tree belonging to the family Lauraceae. *C. cassia* bark or cortex is a natural spice and herb commonly used in traditional medicine to treat dyspepsia, gastritis, blood circulation disturbances, and allergic disease (Leung and Foster, 1996; Nagai, 2004). *C. cassia* has been shown to have many pharmacological properties, such as antiulcerogenic (Akira et al., 1986), antiinflammatory (Atta and Alkofahi, 1998), antipyretic (Kurokawa et al., 1998), antimicrobial (Lee and Ahn, 1998), and antidiabetic activity (Dugoua et al., 2007). It has been also reported that an aqueous extract of *C. cassia* inhibited Arthus allergic reaction in rabbits (Nagai et al., 1982). Novel diterpenes were isolated from bark of *C. cassia* and identified as the active antiallergic substances (Nohara et al., 1982, 1985). Despite traditional usage of *C. cassia* to treat allergic disease, its mechanism has not been fully verified using scientific tools.

Under the circumstances, we investigated the antiallergic effects of *C. cassia* extract on NC/Nga mice as a model of mite antigen-induced atopic dermatitis. We also determined the effect of *C. cassia* on chemokine production in the human keratinocyte cell line HaCaT to examine a possible mechanism for the antiallergic effect of *C. cassia*.

2. Materials and methods

2.1. Preparation of *C. cassia* extract (CCE)

C. cassia as a dried herb was purchased from Omniherb Co. (Yeoungcheon, Korea) and was authenticated based on its microscopic and macroscopic characteristics by The Classification and Identification Committee of the Korea Institute of Oriental Medicine (KIOM). The committee was composed of nine experts in the fields of plant taxonomy, botany, pharmacognosy, and herbolgy. A voucher specimen (no. KIOM109–89A) was deposited at the herbarium of the Department of Herbal Resources Research in KIOM. The dried bark of *C. cassia* (100 g) was extracted twice with 70% ethanol (with 2 h reflux), and the extract was then concentrated under reduced pressure. The decoction was filtered, lyophilized, and serially stored at 4 °C. The yield of dried extract from starting crude materials was about 2.69% (w/w). The extract was dissolved in saline or phosphate-buffered saline (PBS) and then filtered through a 0.22- μ m syringe filter for experiments.

2.2. Experimental animals

Male 8-week-old NC/Nga mice were purchased from SLC, Inc. (Hamamatsu, Japan) and were maintained for 1 week prior to experiments. They were housed in an air-conditioned animal room with a 12-h light/12-h dark cycle at a temperature of $22 \pm 1^\circ\text{C}$ and humidity of $50 \pm 10\%$. Mice were provided with a laboratory diet and water *ad libitum*. All experimental protocols for animal care involving the use of animals were conducted in accordance with National Institutes of Health (NIH) Guidelines and approved by the Committee on Animal Care of our institute.

2.3. Induction of dermatitis

Mice were anesthetized with ether, and the hair on the upper back was shaved with a clipper and a shaver 1 day before experiments. The exposed dorsal region was treated with DfE ointment (Biostir-AD, Biostir, Kobe, Japan) prepared from house dust mites, a crude extract of *D. farinae* (DfE) as described previously (Yamamoto et al., 2007). For topical application, barrier disruption was achieved by treatment with 150 μl of 4% sodium dodecyl sulfate (SDS) on the shaved dorsal skin and both surfaces of each ear 3 h before the DfE ointment application. DfE ointment (100 mg) was then applied to the shaved dorsal skin twice a week for 3 weeks. SDS treatment was carried out prior to each DfE ointment application. On the day of fourth DfE application (day 11), CCE treatment was started. CCE was treated daily for 23 days. Following the last application of CCE (day 33), the mice were killed to investigate immunological and histological changes (day 34). The mice were randomly divided into three groups: (1) normal group without DfE application; (2) control group with DfE application only (100 mg/mouse); (3) CCE-treated group (400 μg /mouse) with DfE application.

2.4. Evaluation of skin severity

The severity of dermatitis was evaluated twice a week, just before the application of DfE and/or CCE. The development of (1) erythema/hemorrhage, (2) dryness/scaling, (3) edema, (4) erosion/excoriation was scored as 0 (none), 1 (mild), 2 (moderate), or 3 (severe). The sum of the individual scores was taken as the dermatitis score (Matsuda et al., 1997). The intensity of score was evaluated as mild (<20%), moderate (20–60%), and severe (>60% of skin area) (Kunz et al., 1997).

2.5. Measurement of serum total IgE, TNF- α , and histamine levels

After blood was collected from the mice on the day of sacrifice, serum samples were obtained by centrifugation ($1700 \times g$, 10 min) and stored at -70°C until use. Total serum IgE levels were measured using an ELISA kit (Shibayagi, Gunma, Japan) according to the manufacturer's instructions. The levels of TNF- α and histamine were also measured using a mouse TNF- α ELISA kit (R&D Systems Inc., Minneapolis, MN, USA) and a histamine ELISA kit (Oxford Biomedical Research Inc., Oxford, MI, USA), respectively.

2.6. Histopathological examination

Dorsal skin and ear samples of NC/Nga mice were removed, fixed in 10% formalin, embedded in paraffin, and serially sectioned at 4 μm . Sections were stained with hematoxylin and eosin solution. In addition, to detect mast cells, sections were stained with toluidine blue. The number of mast cells was counted from the epidermis to the panniculus adiposus, and histological changes were examined by light microscopy.

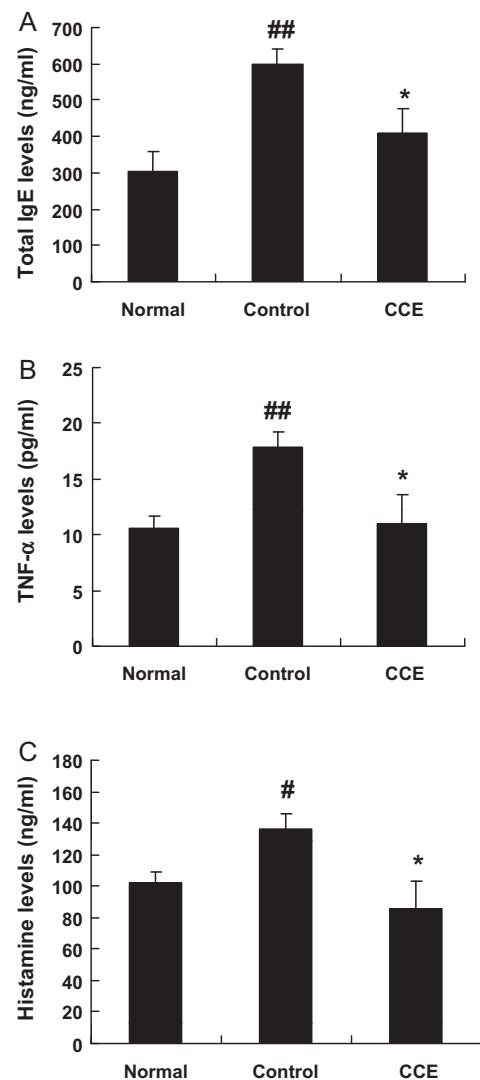


Fig. 2. Effect of CCE on the serum levels of total IgE, TNF- α , and histamine in NC/Nga mice. (A) The levels of IgE, (B) TNF- α , and (C) histamine were measured in serum obtained from animals. Values are expressed as the mean $\pm$ S.E. of 7–8 animals. [#] $P < 0.05$, ^{##} $P < 0.01$ vs. normal group; ^{*} $P < 0.05$ vs. control group.

2.7. Reverse transcriptase-polymerase chain reaction (RT-PCR)

The expression of mRNA transcripts for cytokines and chemokines in the dorsal skin was determined using RT-PCR. The tissue was homogenized, and total RNA was isolated with an easy-BLUE total RNA extraction kit (Intron, Seoul, Korea) according to the manufacturer's instructions. For cDNA synthesis, 1 μg of total RNA was mixed with Maxime RT premix (Intron) containing oligo dT primers and DEPC-treated water to a final volume of 20 μl , and incubated at 45°C for 60 min. The reaction was stopped by heat inactivation at 95°C for 5 min. Then, cDNAs were amplified with primers specific for genes using a Taq PCR master mix kit (Qiagen, Tokyo, Japan) according to the manufacturer's instructions. The 20 μl amplification mixture contained 1 μl of cDNA, 10 μl of $2\times$ taq PCR master mix containing 1.5 mM MgCl_2 , 0.1 μM of each primer, and water. After a 15 min preincubation at 94°C , PCR amplification was performed for 35 cycles with the following steps: denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min. Primers were designed using primer3 software and the sequences available in the GenBank database. The relative expres-

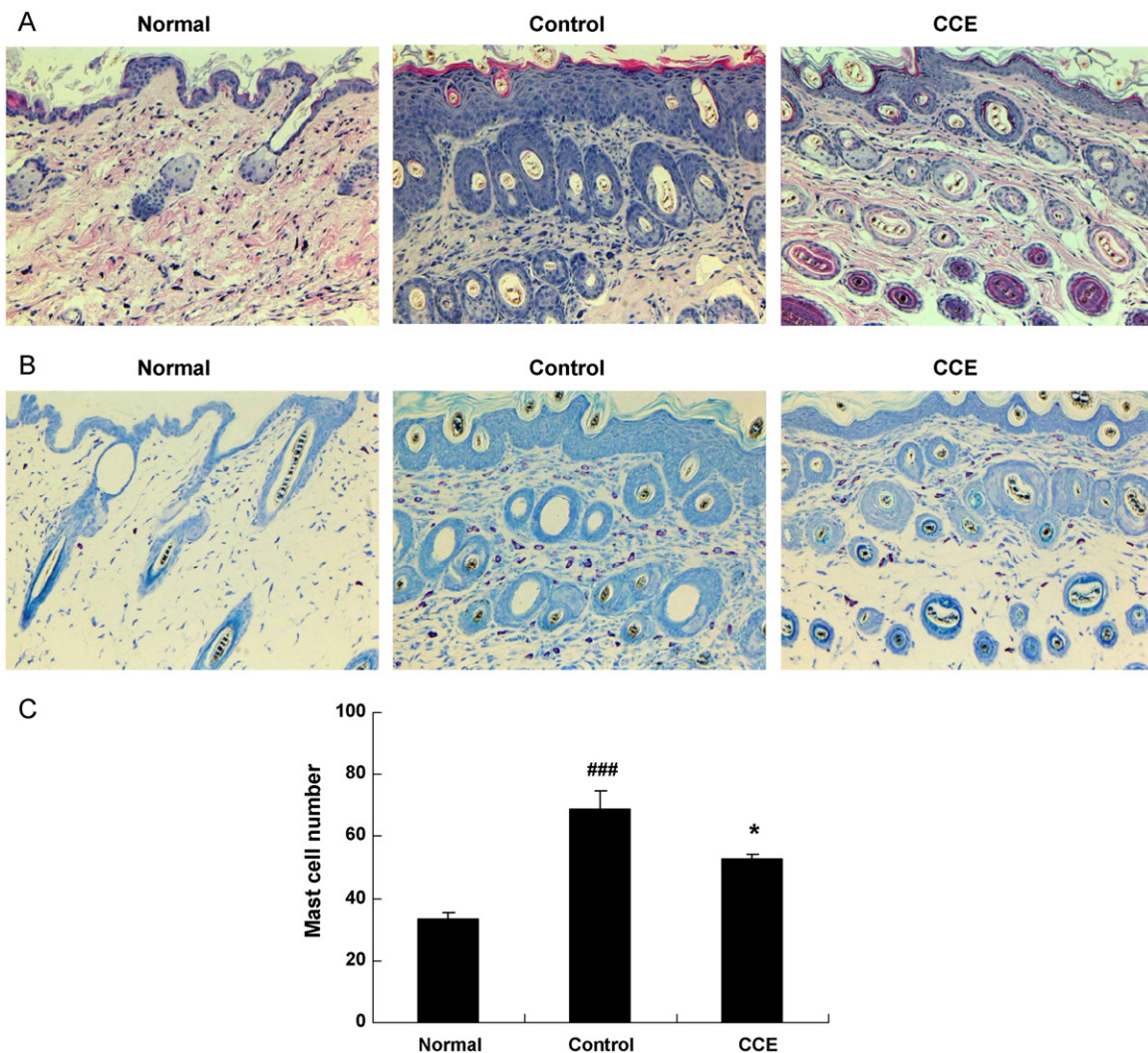


Fig. 3. Histopathologic features of the skin lesions in NC/Nga mice. (A) Hematoxylin and eosin staining. (B) Toluidine blue staining of back skin. (C) Mast cell counts. The mast cells in the tissue sections were stained with toluidine blue and counted with a microscope at a magnification of 400 $\times$. Values are expressed as means $\pm$ S.E. of 7–8 animals. ### $P < 0.001$ vs. normal group; * $P < 0.05$ vs. control group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

sion levels of the target genes were normalized to GAPDH as an internal control.

The primer sequences are as follows: IL-4 (forward) 5'-TCAACCCAGCTAG TTGTCA-3', (reverse) 5'-CATCGAAAAGCCCGAAAGAG-3'; TNF- α (forward) 5'-CCTGTAGCCACGTCGTAGC-3', (reverse) 5'-TTGACCTCAGCGCTGAGTTG-3'; TARC (forward) 5'-CAGGAAGTTGGTGAAGCTGCTATA-3', (reverse) 5'-TTGTGTTCCCTGTAGTGCATA-3'; GAPDH (forward) 5'-TGTTCCTACCCCAATGTGT-3', (reverse) 5'-TGTGAGGGAGATGCTCAGTG-3'.

2.8. Reagents and cell culture

Dulbecco's Modified Eagle's Medium (DMEM), heat-inactivated fetal bovine serum (FBS), penicillin and streptomycin, phosphate-buffered saline (PBS, pH 7.4), and other tissue culture reagents were purchased from Gibco BRL (Grand Island, NY, USA). Human recombinant IFN- γ and human recombinant TNF- α were purchased from R&D Systems Inc.

HaCaT cells were maintained in DMEM supplemented with 10% FBS, 100 μ g/ml streptomycin, and 100 μ g/ml penicillin. HaCaT cells were stimulated with 10 ng/ml human recombinant IFN- γ and 10 ng/ml human recombinant TNF- α in the presence or absence of several doses (0–200 μ g/ml) of CCE for 24 h, as previously described

(Vestergaard et al., 2000). Culture supernatant was collected and stored at -70°C for the ELISA assay.

2.9. ELISA assay for chemokine production in vitro

The amount of TARC, RANTES, and MDC in the culture supernatant of HaCaT cells was determined using a human ELISA kit (R&D Systems Inc.). Briefly, a 96-well plate was plated with captured antibody and blocked with 1% BSA in PBS, after which the supernatants were added and incubated for 2 h at room temperature (RT). After washing, detection antibody was added and incubated for 2 h at RT. After washing, streptavidin–HRP conjugate was added to each well and incubated for 20 min at RT, followed by addition of substrate solution and incubation for 20 min at RT. Finally, stop solution was added, and the optical density of each well was measured at 450 nm using a Benchmark plus microplate spectrophotometer (BioRad, Hercules, CA, USA).

2.10. Statistics

The differences between the treatment groups were examined using an unpaired Student's *t*-test. All data are presented as the mean $\pm$ standard error (S.E.). Significant differences

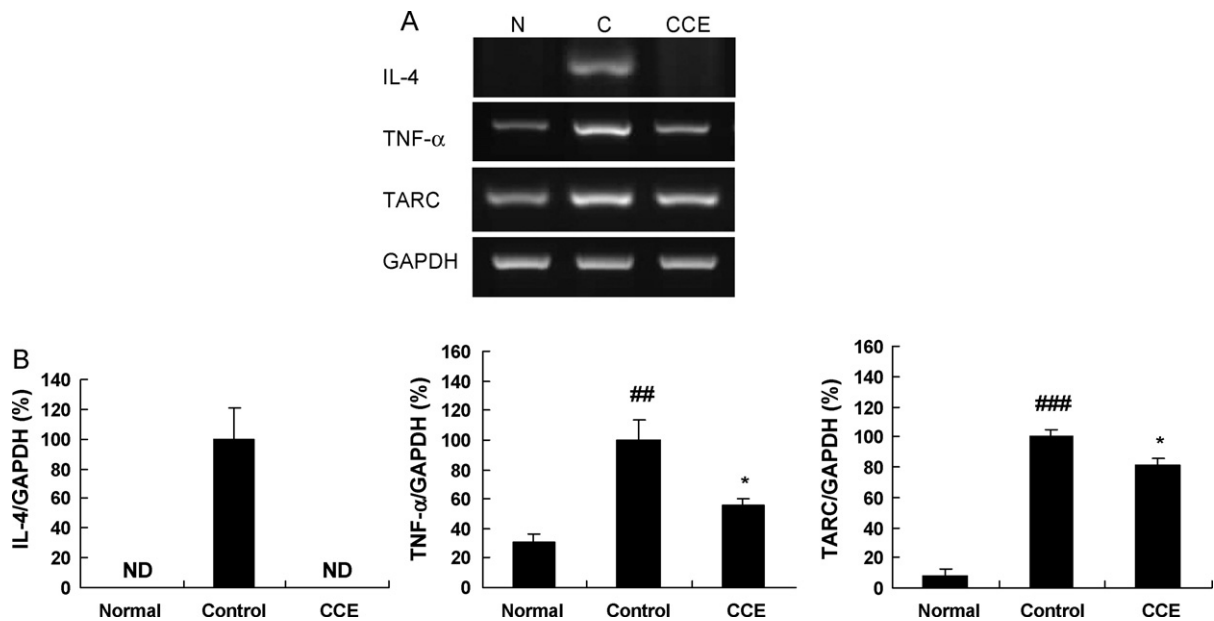


Fig. 4. Effect of CCE on the expression of cytokines and chemokines in the back skin of NC/Nga mice. (A) The mRNA expression of IL-4, TNF- α , TARC was determined. Total RNA was prepared from the back skin of mice treated with DfE and CCE for 34 days, and RT-PCR was performed. Typical results from five independent analyses are shown. (B) The intensity of PCR bands was measured using the NIH ImageJ program. Each data point represents the mean $\pm$ S.E. of five experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. normal group; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ vs. control group.

between groups were accepted when the P -value was less than 0.05.

3. Results

3.1. Effects of CCE on development of dermatitis in NC/Nga mice treated with a mite antigen

To establish the mite antigen-induced dermatitis model in NC/Nga mice, DfE ointment was repeatedly applied twice a week to the dorsal skin. Fig. 1A and 1B show clinical features and the dermatitis severity score in NC/Nga mice treated with the mite antigen. Repeated application of DfE ointment in control mice induced skin dryness first, followed by mild erythema, hemorrhage, and edema (Fig. 1A). Finally, the skin became thick, and severe erythema, hemorrhage, edema, scarring, erosion, and excoriation were observed. However, the application of CCE inhibited the appearance of these skin symptoms. Using the skin severity score, the skin conditions were evaluated twice a week for 34 days after the first application of DfE. As shown in Fig. 1B, the dermatitis score of the control group increased rapidly and became significantly different at 7 days as compared to that of the normal group. However, in the CCE-treated group, the dermatitis score decreased markedly at 14 days compared to that of the control group (Fig. 1B). These results indicate that CCE suppresses the dermatitis spontaneously induced in NC/Nga model mice.

3.2. Effects of CCE on serum IgE, TNF- α , and histamine levels

The serum levels of total IgE, TNF- α , and histamine in NC/Nga mice are shown in Fig. 2A, B, and C. Application of mite antigen for 34 days in the control group considerably enhanced the total serum IgE levels as compared with the normal group, whereas CCE treatment significantly reduced IgE levels (Fig. 2A). Similarly, the proinflammatory cytokine, TNF- α , and histamine levels in the control group increased as compared with those in the normal group, whereas the levels in the CCE-treated group decreased compared to

those in the control group (Fig. 2B and C). These results demonstrate that treatment with CCE prevents the development of dermatitis in NC/Nga mice.

3.3. Histological analysis

Fig. 3A and B show histopathologic features of the skin lesions in NC/Nga mice. Following hematoxylin/eosin staining, hypertrophy and hyperkeratosis of the epidermis, intracellular edema, and infiltration of inflammatory cells were observed in the control group as compared with the normal group. However, treatment with CCE inhibited these inflammatory changes (Fig. 3A).

To investigate whether inflammatory cells had infiltrated into the skin after DfE application, ear and skin tissue sections were stained with toluidine blue to detect mast cells. The number of mast cells in the dermis increased markedly in the control group following DfE application as compared with normal mice, whereas CCE treatment significantly reduced the number of dermal mast cells (Fig. 3B and C).

3.4. Expression of IL-4, TNF- α , and TARC mRNA in the back skin

We investigated the effect of CCE on the mRNA expression of cytokines and chemokines, important markers involved in the pathogenesis of atopic dermatitis, in the back skin of NC/Nga mice. Fig. 4 shows expression of IL-4, TNF- α , and TARC mRNA in the back skin lesions of NC/Nga mice. The mRNA expression of IL-4 increased markedly following DfE application, but expression was undetectable in the normal and CCE-treated groups. We also observed that CCE treatment inhibited DfE-induced expression of the proinflammatory cytokine TNF- α and the Th2 chemokine TARC in the back skin of mice. IFN- γ mRNA expression was also undetectable in both the CCE-treated and the control groups (data not shown). These results reveal that CCE treatment downregulated the level of Th2-specific cytokines and chemokines, leading to the inhibition of skin inflammation mediated by the infiltration of inflammatory cells.

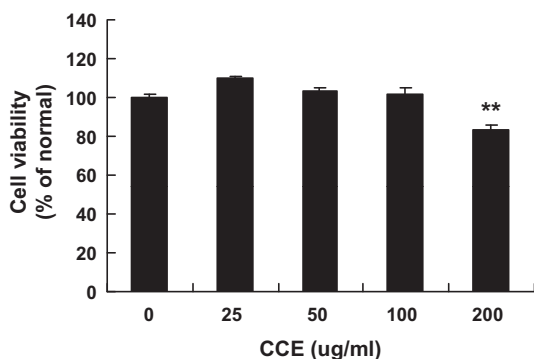


Fig. 5. Effect of CCE on cell viability. HaCaT cells were cultured in 96-well plates and stimulated with or without CCE (0–200 μ g/ml). After 24 h, cell viability was measured using the MTT assay. Each data point represents the mean $\pm$ S.E. of three independent experiments. ** P < 0.01 vs. normal (without CCE).

3.5. Effects of CCE on chemokine production in HaCaT cells treated with IFN- γ /TNF- α

It has been suggested that chemokines including TARC, MDC, and RANTES are involved in the development of Th2-mediated inflammation including atopic dermatitis (Kakinuma et al., 2002; Shimada et al., 2004). Because CCE had a suppressive effect on the development of dermatitis in mite antigen-treated NC/Nga mice, we examined whether CCE affected the production of chemokines in the human keratinocyte cell line HaCaT by stimulation with IFN- γ and TNF- α .

To evaluate the cytotoxic effects of CCE, an MTT assay was performed with the HaCaT cells. The cells were stimulated in the absence or presence of CCE (0–200 μ g/ml) for 24 h. Fig. 5 shows that CCE did not affect cell viability (>80%) and was not toxic to HaCaT cells.

When HaCaT cells were stimulated with 10 ng/ml IFN- γ and 10 ng/ml TNF- α for 24 h, TARC and MDC production increased 14- and 6-fold, respectively, as compared with the vehicle group. Interestingly, in CCE-treated HaCaT cells, the increases in TARC and MDC production were significantly inhibited in a dose-dependent manner (Fig. 6A and B). RANTES production also increased 7-fold in IFN- γ /TNF- α -stimulated HaCaT cells, and its levels were significantly reduced in a dose-dependent manner by treatment with CCE (Fig. 6C). Thus, CCE treatment appears to prevent atopic dermatitis by inhibiting the infiltration of Th2 cells and eosinophils.

4. Discussion

In this study, the antiallergic effects of CCE on NC/Nga mice were evaluated following application of a mite antigen. Topical, repeated application of a mite antigen, DfE ointment, on the back of NC/Nga mice induced atopic dermatitis-like skin lesions with scores of over four, showing itching, skin dryness, mild erythema, edema, and hemorrhage within 2 weeks. The dermatitis scores increased rapidly and became significant by 1 week after the first application. Interestingly, the dermatitis score remained high for at least 2 weeks after the application of DfE ointment was discontinued. At the end of experiment, the skin had become thick, and severe erythema, hemorrhage, edema, erosion, and excoriation were observed. Even though the severity of atopic dermatitis in humans might be different, these clinical symptoms in the dermatitis model were very similar to those of human atopic dermatitis as previously reported (Matsuda et al., 1997; Matsuoka et al., 2003; Yamamoto et al., 2007). In this study, application of CCE on the dorsal skin prevented these skin changes, and dermatitis scores were also reduced. Histological analysis also clearly revealed that the

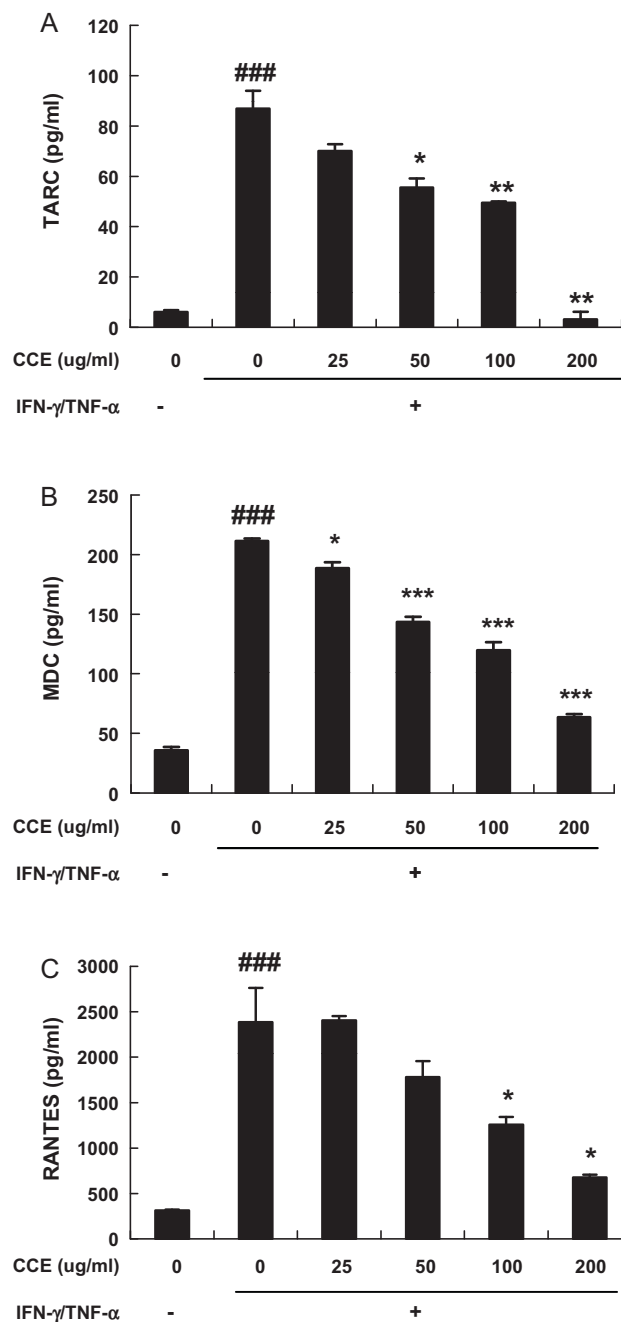


Fig. 6. Effect of CCE on IFN- γ and TNF- α -induced chemokine production in HaCaT cells. HaCaT cells were cultured in 24-well plates and stimulated with recombinant IFN- γ (10 ng/ml) and TNF- α (10 ng/ml) in the presence or absence of CCE (0–200 μ g/ml) for 24 h. Supernatants were collected and measured for TARC, MDC, and RANTES using ELISA kits. Values represent the mean $\pm$ S.E. of three independent experiments. ### P < 0.001 vs. normal (without CCE and IFN- γ /TNF- α); * P < 0.05, ** P < 0.01, *** P < 0.001 vs. IFN- γ /TNF- α alone.

application of CCE inhibited skin inflammation in the NC/Nga mice. Epidermal thickening and infiltration of inflammatory cells including mast cells in the dermis were observed in the control mice, whereas few changes were seen in CCE-treated mice. In addition, body weight gain in the NC/Nga mice was not disrupted by CCE treatment.

Mast cells are strongly associated with immediate hypersensitivity, an immune reaction resulting from the release of histamine, lipid mediators, chemical mediators, and cytokines after mast cell activation through Fc ϵ RI receptor-bound antigen-specific IgE

(Metcalf et al., 1997). IgE-mediated mast cell activation leads to the release of various chemokines and cytokines, which results in infiltration of inflammatory cells into the skin lesions, including eosinophils and lymphocytes. IL-4 exerts switching signals on B cells leading to IgE synthesis. IL-4 is generated and secreted by mast cells and lymphocytes (Kishimoto and Hirano, 1988). Histamine, the main component of granules in mast cells, exerts many effects related to the immediate phase of allergic inflammation, including vasodilation, increased vascular permeability, tissue erythema, contraction of bronchial and gastrointestinal smooth muscle, and increased mucus production (Guo et al., 1997; White, 1999). Mast cell-derived TNF- α has been shown to induce the chemotaxis of neutrophils and T cells, and it is an important mediator of allergic inflammation (Männel and Echtenacher, 2000). Th1-derived cytokines, especially IFN- γ , are strong inhibitors of IgE synthesis, Th2 cell proliferation, and IL-4 receptor expression on T cells (Leung and Bieber, 2003). Previous studies have reported that herbal therapy inhibits the development of atopic dermatitis-like skin lesions in NC/Nga mice by modulating Th1 and/or Th2 cell responses (Park et al., 2007; Kim et al., 2008; Makino et al., 2008). In our current study, serum levels of IgE, histamine, and TNF- α were increased in DfE-treated control mice, but those levels were significantly decreased by the treatment of CCE. IL-4 was not detectable in the serum of NC/Nga mice (data not shown). In addition, mRNA expression of IL-4 and TNF- α , but not IFN- γ , was decreased in the skin lesions of NC/Nga mice with CCE treatment. These data indicate that CCE may suppress the spontaneously induced dermatitis in NC/Nga model mice by suppressing the Th2 cell response.

The infiltration of inflammatory cells to sites of inflammation is known to be dependent on the local production of various members of the chemokine family with leukocyte chemoattractant activity (Oshio et al., 2009). The CC chemokines, such as regulated on activation, normal T cell expressed and secreted (CCL5/RANTES) and CCL11/eotaxin-1 contribute to the chemotaxis of eosinophils expressing CC chemokine receptor 3 (CCR3) and Th2 lymphocytes into the skin. The recruitment of Th2 cells expressing CC chemokine receptor 4 (CCR4) may be mediated by chemokines such as macrophage-derived chemokine (CCL22/MDC) and thymus and activation-regulated chemokine (CCL17/TARC), which are increased in atopic dermatitis. A previous report showed that TARC and MDC are expressed in epidermal keratinocytes and the lesioned skin of NC/Nga mice and human patients with atopic dermatitis (Vestergaard et al., 1999; Shimada et al., 2004; Oshio et al., 2009). Hence, these chemokines, including TARC, MDC, and RANTES, may be important mediators that induce atopic dermatitis via the local accumulation of inflammatory cells. In vitro, RANTES and Th2 chemokines such as MDC and TARC are secreted from the human keratinocyte cell line HaCaT after stimulation with IFN- γ and/or tumor necrosis factor- α (TNF- α) (Vestergaard et al., 2000; Xiao et al., 2003; Kobayashi and Tokura, 2005). In our current study, stimulation of human keratinocytes with IFN- γ and TNF- α increased production of TARC, MDC, and RANTES, and the levels were inhibited in a dose-dependent manner by treatment with CCE. These results imply that CCE treatment can inhibit atopic dermatitis by suppressing the infiltration of Th2 cells and eosinophils into the skin.

5. Conclusion

The topical application of CCE prevented the development of mite antigen-induced atopic dermatitis in NC/Nga mice. We also showed that the inhibition of IL-4, TNF- α , and TARC expression and subsequent inhibition of leukocyte infiltration may be responsible for the inhibitory effect of CCE on atopic dermatitis. The results

from this study suggest that CCE may warrant pilot clinical studies (Phases 1 and 2) on patients with atopic dermatitis.

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References

- Akira, T., Tanaka, S., Tabata, M., 1986. Pharmacological studies on the antiulcerogenic activity of Chinese cinnamon. *Planta Medica* 6, 440–443.
- Atta, A.H., Alkofahi, A., 1998. Anti-nociceptive and anti-inflammatory effects of some Jordanian medicinal plant extracts. *Journal of Ethnopharmacology* 60, 117–124.
- Dugoua, J.J., Seely, D., Perri, D., Cooley, K., Forelli, T., Mills, E., Koren, G., 2007. From type 2 diabetes to antioxidant activity: a systematic review of the safety and efficacy of common and cassia cinnamon bark. *Canadian Journal of Physiology and Pharmacology* 85, 837–847.
- Gao, X.K., Fuseda, K., Shibata, T., Tanaka, H., Inagaki, N., Nagai, H., 2005. Kampo medicines for mite antigen-induced allergic dermatitis in NC/Nga mice. *Evidence-based Complementary and Alternative Medicine* 2, 191–199.
- Guo, Y., Mochizuki, T., Morii, E., Kitamura, Y., Maeyama, K., 1997. Role of mast cell histamine in the formation of rat paw edema: a microdialysis study. *European Journal of Pharmacology* 331, 237–243.
- Horney, B., Steinhoff, M., Ruzicka, T., Leung, D.Y., 2006. Cytokines and chemokines orchestrate atopic skin inflammation. *Journal of Allergy and Clinical Immunology* 118, 178–189.
- Kakinuma, T., Nakamura, K., Wakugawa, M., Mitsui, H., Tada, Y., Saeki, H., Torii, H., Komine, M., Asahina, A., Tamaki, K., 2002. Serum macrophage-derived chemokine (MDC) levels are closely related with the disease activity of atopic dermatitis. *Clinical and Experimental Immunology* 127, 270–273.
- Kim, E.C., Lee, H.S., Kim, S.K., Choi, M.S., Lee, S., Han, J.B., An, H.J., Um, J.Y., Kim, H.M., Lee, N.Y., Bae, H., Min, B.I., 2008. The bark of *Betula platyphylla* var. *japonica* inhibits the development of atopic dermatitis-like skin lesions in NC/Nga mice. *Journal of Ethnopharmacology* 116, 270–278.
- Kishimoto, T., Hirano, T., 1988. Molecular regulation of B lymphocyte response. *Annual Review of Immunology* 6, 485–512.
- Kobayashi, M., Tokura, Y., 2005. Preferential downmodulation of certain chemokines by fexofenadine in human keratinocytes. *Journal of Dermatological Science* 38, 67–69.
- Kunz, B., Oranje, A.P., Labrèze, L., Stalder, J.F., Ring, J., Taïeb, A., 1997. Clinical validation and guidelines for the SCORAD index: consensus report of the European Task Force on atopic dermatitis. *Dermatology* 195, 10–19.
- Kurokawa, M., Kumeda, C.A., Yamamura, J., Kamiyama, T., Shiraki, K., 1998. Antipyretic activity of cinnamyl derivatives and related compounds in influenza virus-infected mice. *European Journal of Pharmacology* 348, 45–51.
- Lee, H.S., Ahn, Y.J., 1998. Growth-inhibiting effects of *Cinnamomum cassia* bark-derived materials on human intestinal bacteria. *Journal of Agricultural and Food Chemistry* 46, 8–12.
- Leung, A.Y., Foster, S., 1996. *Encyclopedia of Common Natural Ingredients Used in Foods, Drugs and Cosmetics*, 2nd ed. John Wiley & Sons, New York, pp. 168–170.
- Leung, D.Y., Bieber, T., 2003. Atopic dermatitis. *The Lancet* 361, 151–160.
- Leung, D.Y., Boguniewicz, M., Howell, M.D., Nomura, I., Hamid, Q.A., 2004. New insights into atopic dermatitis. *The Journal of Clinical Investigation* 113, 651–657.
- Makino, T., Hamanaka, M., Yamashita, H., Mizukami, H., 2008. Effect of bakumijogan, an herbal formula in traditional Chinese medicine, on atopic dermatitis-like skin lesions induced by mite antigen in NC/Jic mice. *Biological and Pharmaceutical Bulletin* 31, 2108–2113.
- Männel, D.N., Echtenacher, B., 2000. TNF- α in the inflammatory response. *Chemical Immunology* 74, 141–161.
- Matsuda, H., Watanabe, N., Geba, G.P., Sperl, J., Tsudzuki, M., Hiroi, J., Matsumoto, M., Ushio, H., Saito, S., Askenase, P.W., Ra, C., 1997. Development of atopic dermatitis-like skin lesion with IgE hyperproduction in NC/Nga mice. *International Immunology* 9, 461–466.
- Matsuoka, H., Maki, N., Yoshida, S., Arai, M., Wang, J., Oikawa, Y., Ikeda, T., Hirota, N., Nakagawa, H., Ishii, A., 2003. A mouse model of the atopic eczema/dermatitis syndrome by repeated application of a crude extract of house-dust mite *Dermatophagoides farinae*. *Allergy* 58, 139–145.
- Metcalf, D.D., Baram, D., Mekori, Y.A., 1997. Mast cells. *Physiological Reviews* 77, 1033–1079.
- Nagai, H., 2004. Immunopharmacological studies on the anti-allergic actions of traditional Chinese medicines and related components. *Journal of Traditional Chinese Medicine* 21, 205–214.
- Nagai, H., Shimazawa, T., Matsuura, N., Koda, A., 1982. Immunopharmacological studies of the aqueous extract of *Cinnamomum cassia* (CCAQ) I. Anti-allergic Action. *Japanese Journal of Pharmacology* 32, 813–822.

- Nohara, T., Kashiwada, T., Nishioka, T., Cinnassiol, E., 1985. A diterpene from the bark of *Cinnamomum cassia*. *Phytochemistry* 24, 1849–1850.
- Nohara, T., Kashiwada, T., Tomimatsu, T., Nishioka, T., 1982. Two novel diterpenes from bark of *Cinnamomum cassia*. *Phytochemistry* 21, 2130–2132.
- Oshio, T., Sasaki, Y., Funakoshi-Tago, M., Aizu-Yokota, E., Sonoda, Y., Matsuoka, H., Kasahara, T., 2009. Dermatophagoides farinae extract induces severe atopic dermatitis in NC/Nga mice, which is effectively suppressed by the administration of tacrolimus ointment. *International Immunopharmacology* 9, 403–411.
- Park, E.J., Park, K.C., Eo, H., Seo, J., Son, M., Kim, K.H., Chang, Y.S., Cho, S.H., Min, K.U., Jin, M., Kim, S., 2007. Suppression of spontaneous dermatitis in NC/Nga murine model by PG102 isolated from *Actinidia arguta*. *Journal of Investigative Dermatology* 127, 1154–1160.
- Shimada, Y., Takehara, K., Sato, S., 2004. Both Th2 and Th1 chemokines (TARC/CCL17, MDC/CCL22, and Mig/CXCL9) are elevated in sera from patients with atopic dermatitis. *Journal of Dermatological Science* 34, 201–208.
- Soter, N.A., 1989. Morphology of atopic eczema. *Allergy* 44, 16–19.
- Spergel, J.M., Paller, A.S., 2003. Atopic dermatitis and the atopic march. *Journal of Allergy and Clinical Immunology* 112, S118–S127.
- 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. *Journal of Investigative Dermatology* 115, 640–646.
- Vestergaard, C., Yoneyama, H., Mural, M., Nakamura, K., Tamaki, K., Terashima, Y., Imai, T., Yoshie, O., Irimura, T., Mizutani, H., Matsushima, K., 1999. Overproduction of Th2-specific chemokines in NC/Nga mice exhibiting atopic dermatitis-like lesions. *The Journal of Clinical Investigation* 104, 1097–1105.
- White, M., 1999. Mediators of inflammation and the inflammatory process. *Journal of Allergy and Clinical Immunology* 103, S378–S381.
- Xiao, T., Kagami, S., Saeki, H., Sugaya, M., Kakinuma, T., Fujita, H., Yano, S., Mitsui, H., Torii, H., Komine, M., Asahina, A., Nakamura, K., Tamaki, K., 2003. Both IL-4 and IL-13 inhibit the TNF- α and IFN- γ enhanced MDC production in a human keratinocyte cell line, HaCaT cells. *Journal of Dermatological Science* 31, 111–117.
- Yamamoto, M., Haruna, T., Yasui, K., Takahashi, H., Iduhara, M., Takaki, S., Deguchi, M., Arimura, A., 2007. A novel atopic dermatitis model induced by topical application with dermatophagoides farinae extract in NC/Nga mice. *Allergology International* 56, 139–148.