

Inhibitory effect of galangin on atopic dermatitis-like skin lesions



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ABSTRACT

Galangin is a member of the flavonol class of flavonoids having anti-inflammatory and anti-oxidative potential. Previously we reported the inhibitory effect of galangin on the mast cell-mediated allergic inflammation. For incremental research, we investigated the effects of galangin on atopic dermatitis (AD)-like skin lesions and underlying mechanisms of action. We established an atopic dermatitis model in BALB/c mice by repeated local exposure of house dust mite (*Dermatophagoides farinae*) extract (DFE) and 2,4-dinitrochlorobenzene (DNCB) to the ears. Repeated alternative treatment of DFE/DNCB caused AD-like skin lesions. Topical application of galangin reduced AD symptoms based on ear thickness and histopathological analysis, in addition to serum IgE and IgG2a levels. Galangin inhibited mast cell infiltration into the ear and serum histamine level. Galangin suppressed DFE/DNCB-induced expression of interleukin (IL)-4, IL-5, IL-13, IL-31, IL-32, and interferon (IFN)- γ in the ear tissue. To define the underlying mechanisms of action, tumor necrosis factor- α /IFN- γ -activated human keratinocytes (HaCaT) model was used. Galangin significantly inhibited the expression of cytokines and chemokine by the down-regulation of nuclear factor- κ B and mitogen-activated protein kinases in HaCaT cells. Taken together, the results demonstrate that galangin inhibited AD-like symptoms, suggesting that galangin might be a candidate for the treatment of AD.

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1. Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease associated with a combination of intense pruritus, scratching, and cutaneous sensitization with allergens (Jin et al., 2009b). Pathophysiology of AD is complex and regulated by a multitude of genetic and environmental factors (Novak and Simon, 2011). Among environmental factors, house dust mite allergens are important for the development of AD, and *Dermatophagoides farinae* extract (DFE) is routinely used for the experimental induction of AD (Choi et al., 2013; Dai et al., 2011; Kwon et al., 2010). House dust mite allergens are known to contribute to pathogenesis of AD through the induction of Th2 cells to produce interleukin (IL)-4 and IL-13 (Guttman-Yassky et al., 2011b; Thomas et al., 2002). These cytokines induce immunoglobulin (Ig) E class switching and promote Th2 cell survival. Numbers of Th2 cells are elevated during the acute and chronic stages of AD. In addition, the cytokines they produce (IL-4 and IL-13) have direct effects on the epidermis, such as inducing keratinocytes to produce various proinflammatory

cytokines and chemokines. These conditions are characterized by infiltrates of T cells, monocytes, macrophages, eosinophil and mast cells (Zimmermann et al., 2011). Mast cells have important roles in inflammation, and regulate eosinophil activation and recruitment (Guttman-Yassky et al., 2011a). Mast cell-derived histamine and inflammatory mediators contribute to itching and inflammation in AD (Oyoshi et al., 2009).

The pharmacological control of AD is an important clinical issue. A large number of studies have dealt with the effect of topical or systemic administration of corticosteroids, anti-histamines and calcineurin inhibitors (Quemeneur et al., 2003). However, it is well known that prolonged use of glucocorticoids and immune suppressors causes a variety of side effects. Recently, several effective therapies and agents involving natural materials have been introduced for AD treatment. Galangin (3,5,7-trihydroxyflavone) is a member of the flavonol class of flavonoids, and is present at high concentration in propolis, a natural material produced in honey and *Alpinis officinarum*, a plant which is a common herbal medicine in Asia (Heo et al., 2001). Galangin possesses certain pharmacological activities, including anti-mutagenic, anti-oxidative, and radical scavenging (Cushnie and Lamb, 2006; Gwak et al., 2011; Heo et al., 2001). We previously reported that galangin inhibited mast cell-derived allergic inflammation (Kim et al., 2013). Galangin also

Abbreviations: AD, atopic dermatitis; DFE, *Dermatophagoides farinae* extract; DNCB, 2,4-dinitrochlorobenzene.

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showed anti-inflammatory effects on collagen-induced arthritis mice without toxicity via attenuation of RANKL-induced activation of JNK, p38 and NF- κ B pathways (Huh et al., 2013). However, the role of galangin on AD has not been studied yet. The objective of this study was to elucidate the effects of galangin on the AD-like lesions and to define the underlying mechanisms of these effects.

2. Materials and methods

2.1. Animals

Six-week-old female BALB/c mice were purchased from SLC Inc. (Hamamatsu, Japan). The animals were housed with 5–10 mice per cage in a laminar air flow room maintained at a temperature of $22 \pm 2^\circ\text{C}$ with a relative humidity of $55 \pm 5\%$ throughout the study. The care and treatment of the mice were in accordance with the guidelines established by the Public Health Service Policy on the Humane Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

2.2. Drugs and chemicals

Galangin was purchased from Sigma (St. Louis, MO). *Dermatophagoides farinae* extract (DFE, Greer Laboratories, Lenoir, NC) was used as an antigen. All other reagents were purchased from Sigma unless otherwise stated. DFE was dissolved in phosphate-buffered saline (PBS) containing 0.5% Tween 20. 2,4-Dinitrochlorobenzene (DNCB, 1%) was dissolved in an acetone/olive oil (1:3) solution. DMEM, antibiotics, and trypsin–EDTA were obtained from Invitrogen (Grand Island, NY). Recombinant human TNF- α and IFN- γ were purchased from R&D systems (Minneapolis, MN).

2.3. Cell culture and viability

A human keratinocyte cell line, HaCaT, was maintained in DMEM supplemented with 10% FBS and antibiotics (100 U/mL penicillin G, 100 $\mu\text{g/mL}$ streptomycin) at 37°C in 5% CO_2 . Cell viability was determined using the 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide assay (MTT, Sigma). After 24 h of treatment, MTT (5 mg/mL) was added into each well that contained a sample and incubated for 2 h. Isopropanol was added to dissolve the formazan crystals. Absorbance on each sample compared to that of the control, was calculated and expressed as percentage.

2.4. Induction of AD-like lesions in the mouse ear

The induction of AD-like lesions by DFE and DNCB was performed based on our previous research (Choi et al., 2013; Kwon et al., 2010). A schematic experimental procedure is described in Fig. 1A. Mice ($n = 5$) were divided into four groups, and the surfaces of both ear lobes were stripped five times with surgical tape (Nichiban, Tokyo, Japan). After stripping, 20 μL of DNCB (1%) was painted on each ear and then 20 μL of DFE (10 mg/mL) 4 days later. Treatment of DFE/DNCB was alternatively applied to both ears once a week. Two weeks after the first induction, tail bleeding was performed to check the serum IgE level. After confirming an atopic condition by IgE level, ears were treated with galangin (1 or 5 $\mu\text{g/ear}$) by painting until the end of 4 weeks induction. Ear thickness was measured 24 h after DFE or DNCB application with a dial thickness gauge (Mitutoyo, Co., Tokyo, Japan).

On day 28, blood samples were collected by an orbital puncture. The plasma was stored at -70°C for further analysis. After blood collection, ears were removed and used for a histopathological analysis. Serum IgE and IgG2a levels were measured using an ELISA kit (BD Biosciences, Oxford, UK) according to the manufacturer's instructions. Briefly, for the detection of total IgE or IgG2a, 96-well plates (Nunc, Wiesbaden, Germany) were first coated overnight with anti-mouse IgE or anti-mouse IgG2a antibody. After blocking of unspecific binding, detection was performed using biotinylated anti-mouse IgE or anti-mouse IgG2a antibodies and streptavidin conjugated HRP. The colorimetric reaction of peroxidase substrate (TMB; KPL, Gaithersburg, MD) was measured at 450 nm after addition of 1 M sulphuric acid. Diluted mouse immunoglobulin was used for standard curve calculation. For the detection of DFE-specific IgE, 10 mg of mite extract was coated in 96 well plates. The DFE-specific IgE level was indicated by the O.D. value.

2.5. Histological observation

The ears were fixed with 10% formaldehyde and embedded in paraffin. Thin 5 μm sections were stained with hematoxylin and eosin. Infiltrated lymphocytes, thickening of the epidermis, and fibrosis in the dermis were observed by microscope. For measurement of mast cell infiltration, skin sections were stained with toluidine blue, and the number of mast cells in five sites chosen at random was counted. Eosinophils were counted blinded in 10 high-power fields at a magnifica-

tion of $400\times$. Dermal thickness was analyzed in H&E-stained sections viewed under a magnification of $100\times$. Thickness was measured in five randomly selected fields from each sample.

2.6. Histamine assay

Histamine content was measured through the *o*-phthalaldehyde spectrofluorometric procedure based on previous research (Bae et al., 2011). The blood from the mice was centrifuged at 400g for 10 min, and the serum was withdrawn to measure histamine content. Fluorescence intensity was measured using 355 nm excitation and 450 nm filters and the fluorescence Spectrometer LS-50B (Perkin–Elmer, Norwalk, CT).

2.7. Real-time polymerase chain reaction (PCR)

For the detection of expression of cytokines, quantitative real-time PCR was carried out using the Thermal Cycler Dice TP850 (Takarabio Inc., Shiga, Japan) according to the manufacturer's protocol. At the end of *in vivo* experimental period, the ears were excised, and total RNA was isolated. HaCaT cells were pretreated with galangin for 1 h, and then stimulated with TNF- α (10 ng/mL) plus IFN- γ (10 ng/mL) for 6 h. Total cellular RNA was isolated from cells (2×10^5 cells/well in 24-well plate) as described in previous research (Lee et al., 2011). Briefly, 2 μL of cDNA (100 ng), 1 μL of sense and antisense primer solution (0.4 μM), 12.5 μL of SYBR Premix Ex Taq (Takarabio Inc., Shiga, Japan), and 9.5 μL of dH_2O were mixed together to obtain a final 25 μL reaction mixture in each reaction tube. The conditions for PCR were similar to ones as described in previous research (Choi et al., 2011) except for the primer of mIFN- γ (F 5'-TCA AGT GGC ATA GAT GTG GAA GAA-3', R 5'-TGG CTC TGC AGG ATT TTC ATG-3') and mIL-32 (F 5'-GCC CAA GAT CGT CAA AAA GA-3', R 5'-ATT GTG GAC CAG GAA CTT GC-3'). The normalization and quantification of mRNA expression was performed using the TP850 software supplied by the manufacturer.

2.8. Nuclear protein extraction

Samples for nuclear protein extraction were prepared as described in previous research (Kim et al., 2014). HaCaT cells were pretreated with galangin for 1 h, and then stimulated with TNF- α (10 ng/mL) plus IFN- γ (10 ng/mL) for 20 min. After stimulation, cells (2×10^6 cells/well in 6-well plate) were washed in 1 mL of ice-cold PBS, centrifuged at 1200g for 5 min, resuspended in 400 μL of ice-cold hypotonic buffer (10 mM HEPES/KOH, 2 mM MgCl_2 , 0.1 mM EDTA, 10 mM KCl, 1 mM DTT, 0.5 mM PMSF, pH 7.9), left on ice for 10 min, vortexed, and centrifuged at 15,000g for 30 s. After washing, pelleted nuclei were resuspended in 50 μL of ice-cold saline buffer (50 mM HEPES/KOH, 50 mM KCl, 300 mM NaCl, 0.1 mM EDTA, 10% glycerol, 1 mM DTT, 0.5 mM PMSF, pH 7.9), left on ice for 20 min, vortexed, centrifuged at 15,000g for 5 min at 4°C , and supernatant gathered.

2.9. Western blot

Samples for Western blot were prepared as described in previous research (Lee et al., 2011). Briefly, HaCaT cells were pretreated with galangin for 1 h, and then stimulated with TNF- α (10 ng/mL) plus IFN- γ (10 ng/mL) during 20 min for MAPKs. Cells (2×10^6 cells/well in 6-well plate) were rinsed twice with ice-cold PBS, and total cell lysates were gathered in 200 μL of lysis buffer. The lysates were spun in a micro-centrifuge for 20 min at 4°C , and the supernatant was collected. Proteins were electrophoresed using 8–12% SDS–PAGE, and then transferred to nitrocellulose membranes. The membranes were stained with reversible Ponceau S to ascertain equal loading of samples in the gel. Immunodetection was performed using an enhanced chemiluminescence detection kit (Amersham, Piscataway, NJ).

2.10. Statistical analysis

Statistical analyses were performed using Prism 5 (GraphPad Software, San Diego, CA). Treatment effects were analyzed using one way analysis of variance followed by Dunnett's test. A value of $p < 0.05$ was used to indicate statistically significant differences.

3. Results

3.1. Effects of galangin on the ear thickness and histopathological observation

To determine the effect of galangin on AD-like skin lesions, galangin was treated on the ears of BALB/c mice with DFE/DNCB every day during a 3-week period. The experimental procedure of AD-like skin lesions was shown in Fig. 1A. DFE/DNCB treatment for 4 weeks elicited severe AD-like skin lesions. During the induction period, we measured ear swelling 24 h after each induction. The

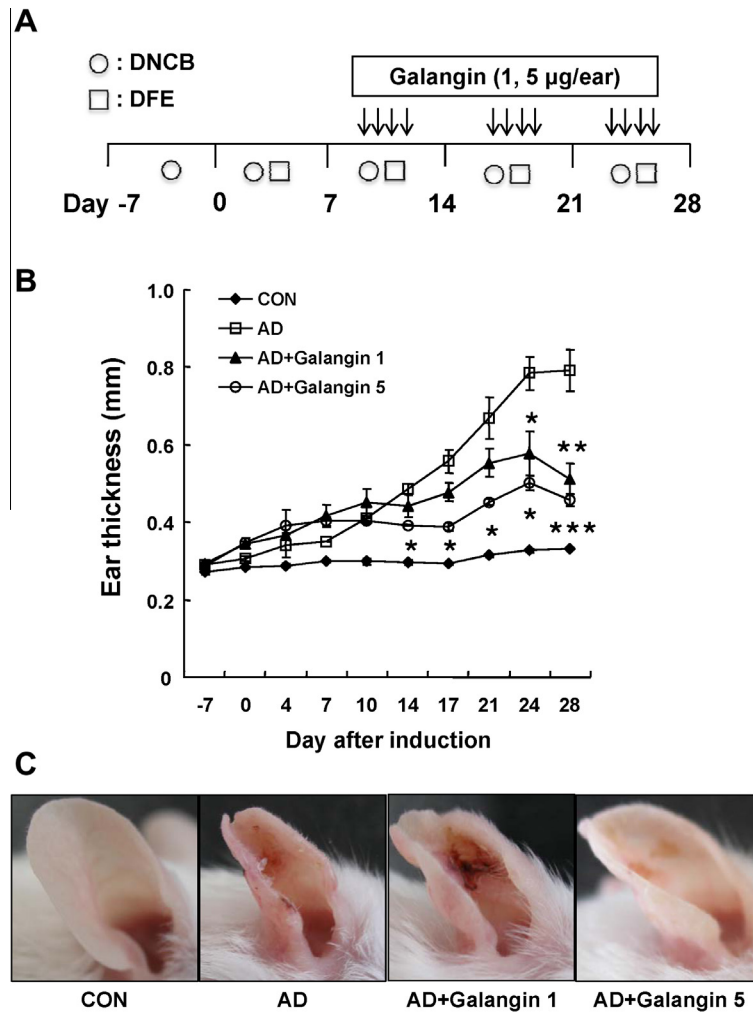


Fig. 1. Effects of galangin on ear thickness in AD mice. (A) Experimental design for the induction of AD. One week after the first boost with 1% DNCB (20 μ L/ear), DFE (10 mg/mL, 20 μ L/ear) and DNCB was alternatively applied to both ears once a week. After 2 weeks of induction, galangin (1, 5 μ g/ear) was treated every day for 3 weeks ($n = 5$). (B) Ear thickness was measured 24 h after DFE or DNCB application with a dial thickness gauge. Data are presented as mean $\pm$ SD of five determinations. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ significantly lower than AD. (C) Representative photographed example of the effects of galangin on DFE/DNCB-induced AD in the ear.

tendency of ear swelling of each group was similar until 2 weeks. After 3 weeks of AD-like skin lesion induction, galangin treated groups showed a significant improvement of ear thickness and photograph (Fig. 1B and C).

Microscopic analysis of the ear skin showed many alterations. These features, including hyperkeratosis, large ulcers, and acute inflammatory cell infiltration, were observed (Fig. 2A upper panel). Compared with AD-like skin lesions, galangin significantly decreased DFE/DNCB-induced epidermal and dermal thickness (Fig. 2B) and infiltration of eosinophils (Fig. 2C). Mast cells are important group of effector cells and a source of histamine in AD. Histamine is a representative symptom-inducing substance in AD (Guttman-Yassky et al., 2011a). Therefore, the infiltration of mast cells in AD site and the serum level of histamine were examined. Galangin inhibited the infiltration of mast cells (Fig. 2A lower panel and 2D) and serum histamine (Fig. 3A).

3.2. Effects of galangin on serum immunoglobulin and expression of cytokines

IgE production is associated with Th2 cellular response. On the contrary, IgG2a is associated with Th1 response (Bieber, 2010). To discriminate the role of galangin on the Th1 or Th2 response, we examined serum levels of IgE (total and DFE specific) and IgG2a.

Compared with the AD, total IgE, DFE-specific IgE, and IgG2a were significantly reduced by the galangin (Fig. 3B–D).

The acute phase of AD is predominantly a Th2 response, while the chronic phase is characterized by Th1 response (Bieber, 2010; Oyoshi et al., 2009). We examined the effect of galangin on the expression of AD-related inflammatory cytokines from the ear tissue by the real-time PCR. All cytokines were upregulated in AD-like skin lesions, and galangin inhibited the expression of both Th1 (IFN- γ) and Th2 (IL-4, IL-5, IL-13, IL-31 and IL-32) cytokines (Fig. 4). These results suggest that galangin inhibits expression of both Th1 and Th2 cytokines in AD-like skin lesions. In addition, galangin plays a suppressive role in chronic skin inflammation elicited by AD-like skin lesions.

3.3. Effects of galangin on the activation of keratinocytes

After the observation of inhibitory effect of galangin on the AD models, a human keratinocyte model was used for clarifying the biological function and molecular mechanism of the galangin. Keratinocytes have a crucial role on immune responses during development of AD-like skin lesions. Keratinocytes produce inflammatory factors that promote chronic, self-amplifying loops of immune activation (Guttman-Yassky et al., 2011a). Therefore, TNF- α /IFN- γ -induced pro-inflammatory cytokines (TNF- α , IL-1 β

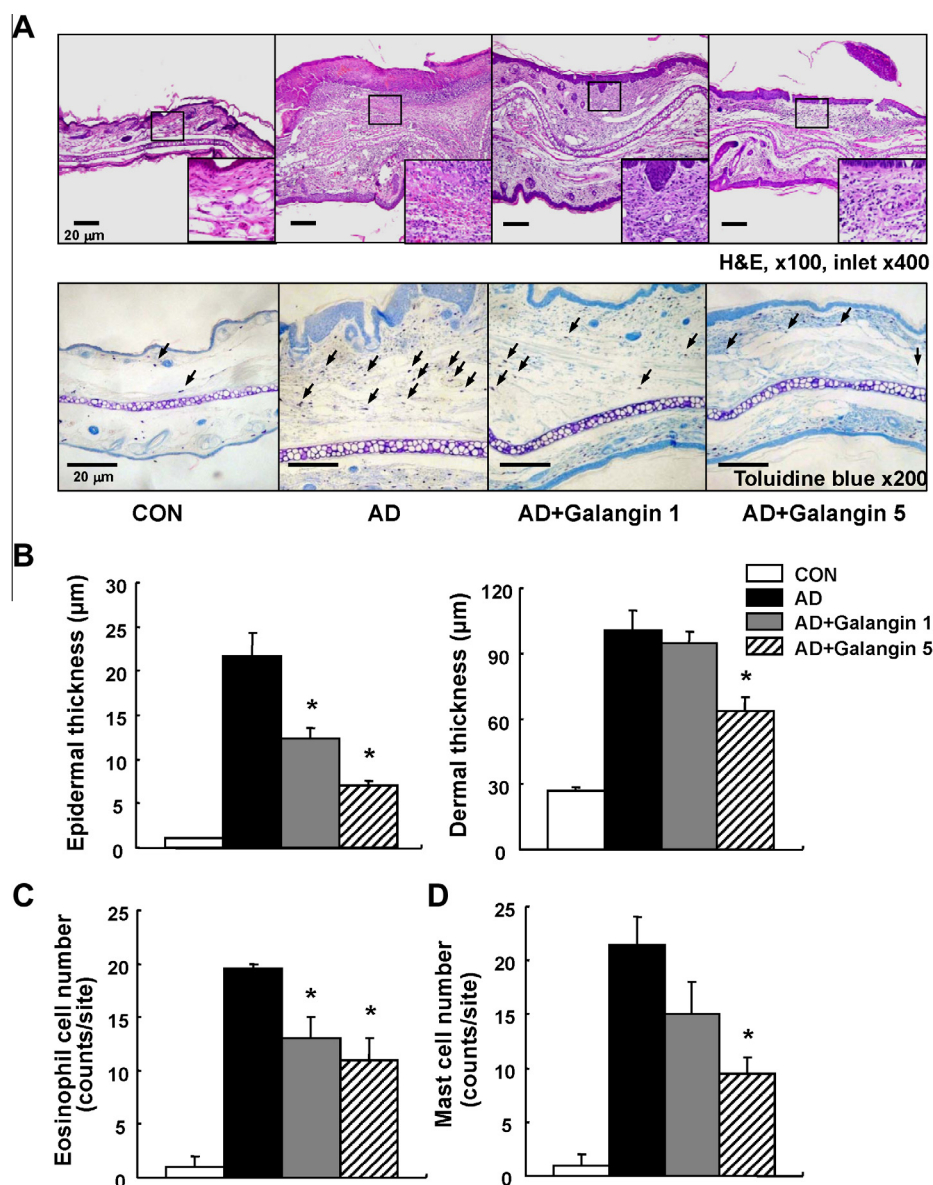


Fig. 2. Histological analysis of AD mice. (A) Representative photomicrographs of ear sections were stained with hematoxylin and eosin (upper panel, original magnification 100×) or toluidine blue (lower panel, original magnification 200×). (B) Epidermal and dermal thickness. (C) Numbers of eosinophils. (D) Numbers of mast cells. Scale bar = 20 μm. Data are presented as mean ± SD of five determinations. **p* < 0.05 significantly lower than AD.

and IL-6) and the chemokine CCL17 in keratinocytes are considered as the most important mediators for the development of AD (Vestergaard et al., 2000). HaCaT cells were pretreated with galangin for 1 h and then stimulated with TNF-α/IFN-γ for 6 h. The results of real-time PCR showed that galangin inhibited TNF-α/IFN-γ-induced gene expression of TNF-α, IL-1β, IL-6 and CCL17 in HaCaT cells (Fig. 5A). These results suggest that galangin mitigated the inflammatory response by the down-regulation of the pro-inflammatory cytokines and chemokine in AD-like skin lesions.

TNF-α/IFN-γ-induced pro-inflammatory cytokines and CCL17 are regulated by MAPKs and NF-κB (Kim et al., 2011). To confirm the role of MAPKs and NF-κB on the expression of TNF-α/IFN-γ-induced cytokine and chemokine, specific inhibitors for ERK (PD98059), p38 (SB203580), JNK (SP600125) and NF-κB (PDTC) were used. As shown in Fig. 5B, specific inhibitors for ERK, p38 and NF-κB significantly inhibited TNF-α/IFN-γ-induced CCL17 expression. However, inhibitor for JNK appeared not to inhibit CCL17 in HaCaT cells. Galangin did not show cytotoxicity upto 10 μg/mL in keratinocytes (Fig. 6A).

To investigate the mechanism responsible for the inhibitory effect of galangin on the cytokine expression, we investigated the effect of galangin on the TNF-α/IFN-γ-induced MAPKs and NF-κB. HaCaT cells were pretreated with galangin for 1 h, and then stimulated with TNF-α/IFN-γ for 20 min. As shown in Fig. 6B, treatment of TNF-α/IFN-γ showed activation of MAPKs and NF-κB. Galangin (10 μg/mL) decreased TNF-α/IFN-γ-induced phosphorylation of ERK and p38, and nuclear translocation of p65 NF-κB following degradation of IκBα. However, galangin did not reduce the activation of JNK. These results suggest that galangin might suppress TNF-α/IFN-γ-induced cytokines and chemokine by the reduction of ERK, p38 and NF-κB in keratinocytes.

4. Discussion

Propolis is a resinous mixture that honey bees collect from various plants. Several studies have demonstrated that propolis might act as potent anti-inflammatory agent against both acute and

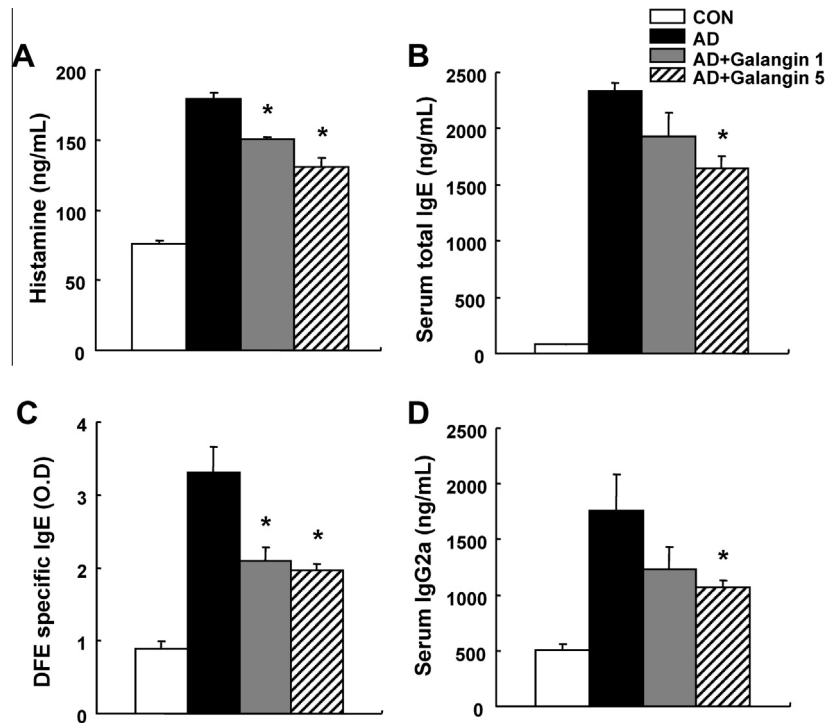


Fig. 3. Serum levels in AD mice. The blood samples of vehicle, DFE/DNCB plus vehicle, and DFE/DNCB plus galangin (1, 5 μ g/ear) groups were collected by orbital puncture at 28 days, and serum level was measured by ELISA. Data are presented as mean $\pm$ SD of five determinations. * $p < 0.05$ significantly lower than AD.

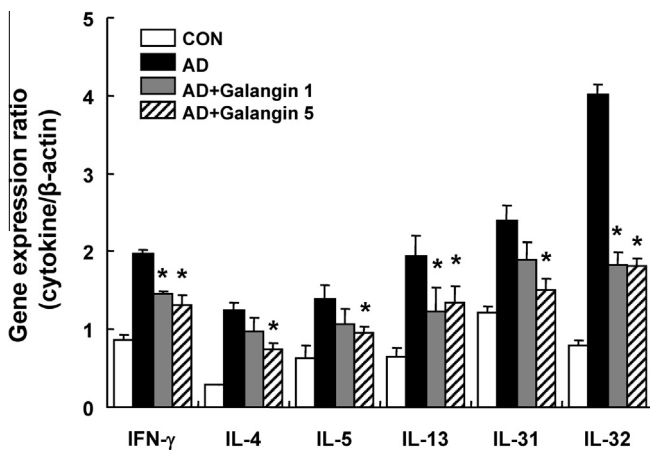


Fig. 4. Expression of cytokines in the ear of AD mice. To determine cytokine levels in AD mice, ears were excised on day 28. Total RNA was isolated from the ear using the Trizol reagent, and mRNAs for various cytokines were analyzed by the real-time PCR. Data are presented as mean $\pm$ SD of five determinations. * $p < 0.05$ significantly lower than AD.

chronic inflammation (Borrelli et al., 2002; Khayyal et al., 1993). Propolis is generally composed of flavonoids, phenolic acid, phenolic aldehydes, ketone, wax, and resin. Among them, chrysin and galangin have been identified as active components of propolis (Gomez-Caravaca et al., 2006). Based on the known anti-allergic and anti-inflammatory effects of propolis, we examined the role of galangin in AD-like skin lesions using *in vivo* and *in vitro* models.

Acute AD lesions exhibit spongiosis (epidermal intercellular edema), hyperkeratosis (thickening of the stratum corneum), and chronic lesions characterized by acanthosis (diffuse epidermal hyperplasia) and infiltration of lymphocytes and mast cells (Kawakami et al., 2009). In the present report, galangin mitigated typical and histological changes, such as severe ear thickness, ulcers, dermal and epidermal thickness, epidermal hyperplasia and

infiltration of inflammatory cells (eosinophils and mast cells). Mast cells release a number of important signaling molecules, among which histamine has particularly potent pro-inflammatory activities (O'Mahony et al., 2011). Histamine mainly induces erythema and edema, and is likely to be a significant mediator in patients with AD (Kawakami et al., 2009). In addition, serum histamine levels were reported to be significantly higher in patients with AD than controls (Greaves, 2005; Herman and Vender, 2003). In our results, treatment with galangin reduced serum histamine and pathogenesis of the skin lesions in AD. According to our results, we speculate that galangin has inhibitory effect on the development of AD by the reduction of histamine following the decrease of edema.

In AD patients, elevated total IgE and specific IgE to environmental allergens can be detected (Schmid-Grendelmeier et al., 2001). Langerhans cells of AD skin bearing the high-affinity IgE receptor Fc ϵ RI on their surface provide a link between environmental allergen exposure and Th2 cell activation (Dokmeci and Herrick, 2008). Th2 cell activation evokes expression of IL-4, IL-5, and IL-13 which regulating IgE class-switching by B cells (Dokmeci and Herrick, 2008). Levels of IL-31 also correlate with levels of Th2 cells in the skin of subjects with AD (Neis et al., 2006). IL-31 transgenic mice developed spontaneous pruritus and hallmarks of AD skin lesions (Brandt and Sivaprasad, 2011). In addition, it is known that IFN- γ drives the production of IgG2a (Jin et al., 2009a). In our AD model, galangin markedly inhibited the expression of Th1 (IFN- γ) and Th2 (IL-4, IL-5, IL-13, and IL-31) cytokines, and serum level of IgE and IgG2a. These results imply that galangin suppressed both Th1 and Th2 responses. Recently, it has been reported that IL-32 is expressed in lesional skin of AD patients, and contributes to the pathophysiology of AD (Meyer et al., 2010). In our results, galangin markedly inhibited DFE/DNCB-induced Th1 and Th2 cytokines and IL-32 in ear tissue. These results imply that galangin reduced both acute and chronic AD symptoms. Furthermore, our data suggest that IL-32 is a target of galangin in AD.

Keratinocytes produce cytokines and chemokines which are involved in the development of inflammatory skin disorders

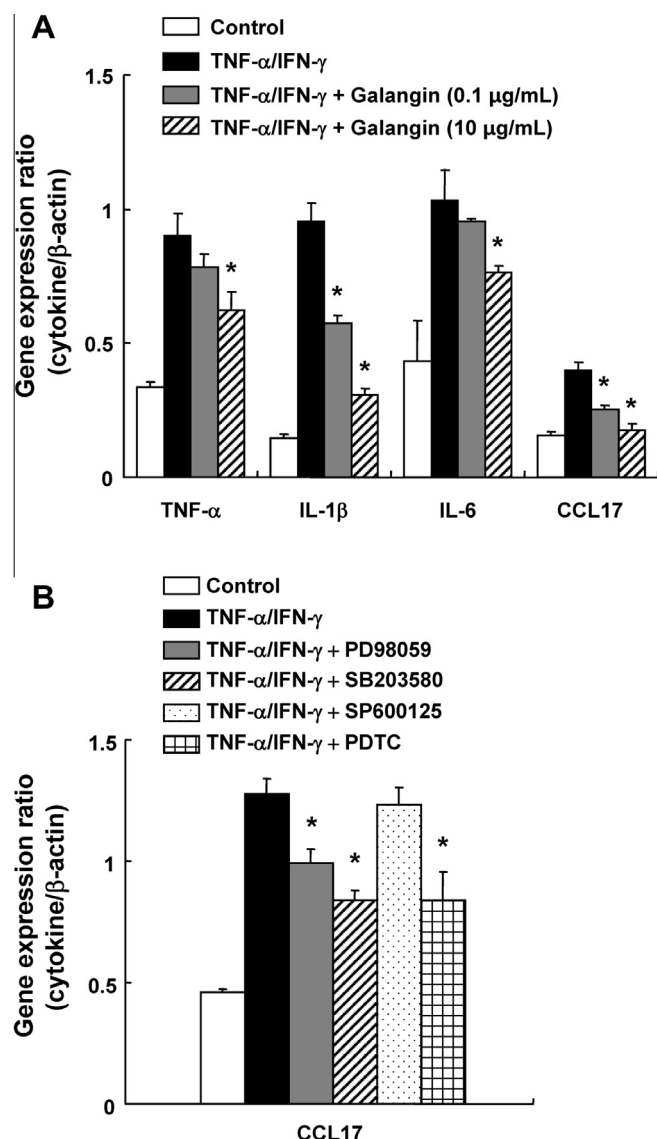


Fig. 5. Effects of galangin on TNF- α /IFN- γ -stimulated HaCaT cells. Cells were pretreated with galangin (0.1, 10 μ g/mL) or specific inhibitors 1 h before the stimulation with TNF- α (10 ng/mL) plus IFN- γ (10 ng/mL) during 6 h. (A) Expression levels of TNF- α , IL-1 β , IL-6, and CCL17 (B) Specific inhibitors for ERK (PD98059, 20 μ M), p38 (SB203580, 10 μ M), JNK (SP600125, 5 μ M) and NF- κ B (PDTC, 10 μ M) were used. Expression levels of cytokines and chemokine were determined by the real-time PCR. Data are presented as mean $\pm$ SD. * p < 0.05 significantly lower than stimulated TNF- α /IFN- γ positive control.

especially AD (Kwon et al., 2012). TNF- α and IFN- γ synergistically induce cytokines for AD symptoms in keratinocytes, and this experimental model has been widely used for imitating AD symptoms *in vitro* (Leung and Bieber, 2003; Leung et al., 2004; Vestergaard et al., 2000). In our results, galangin suppressed TNF- α /IFN- γ -induced CCL17, IL-6, TNF- α and IL-1 β . Recently, we demonstrated that galangin inhibits gene expression of TNF- α , IL-1 β and IL-6 in mast cells (Kim et al., 2013). These accumulated reports support our results showing the diminution of pro-inflammatory cytokines in keratinocytes. CCL17 and cytokine production in keratinocytes is upregulated by TNF- α /IFN- γ through the NF- κ B pathway (Kwon et al., 2012; Saeki and Tamaki, 2006). Galangin inhibited the degradation of I κ B α and nuclear translocation of NF- κ B.

The MAPK cascade is also an important signaling pathway in immune responses and cross talks with NF- κ B to regulate inflammation signaling (Arbabi and Maier, 2002). Previous studies

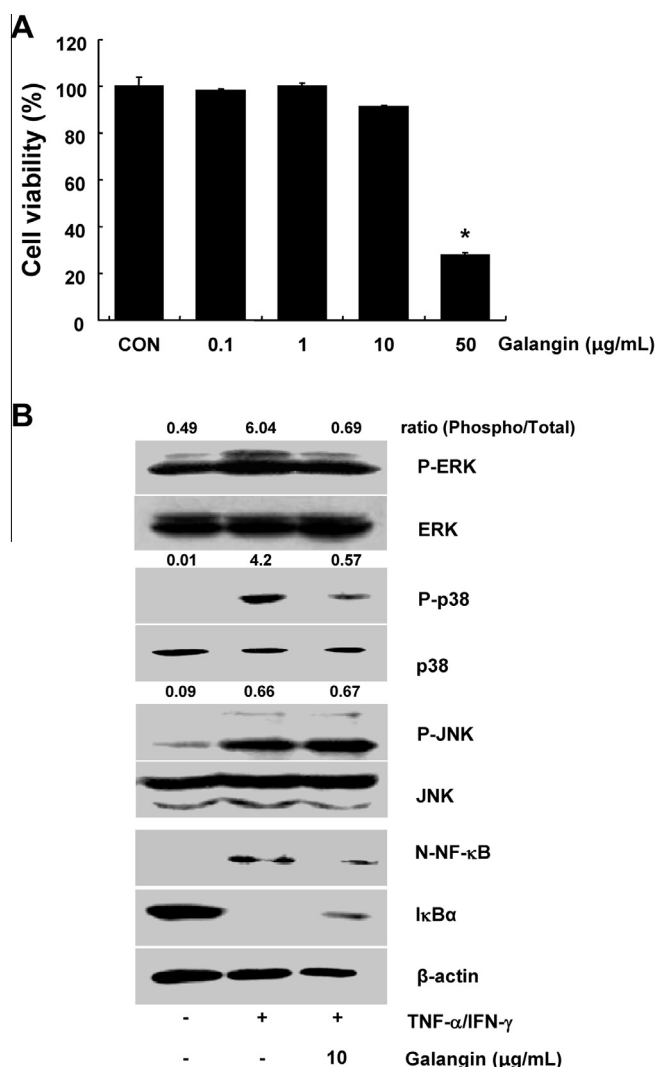


Fig. 6. Effects of galangin on cell viability and the activation of MAPKs and NF- κ B. (A) Cell viability was determined using the MTT assay during 24 h. Data are presented as mean $\pm$ SD of five determinations. * p < 0.05 lower than control. (B) Cells were pretreated with galangin (10 μ g/mL) 1 h before stimulation with TNF- α (10 ng/mL) plus IFN- γ (10 ng/mL) for 20 min. The phosphorylation of MAPKs, the degradation of I κ B α , and the nuclear translocation of NF- κ B were analyzed by the Western blot. The experiment shown is representative of three independent experiments. N-NF- κ B, nucleus NF- κ B.

reported that TNF- α /IFN- γ -stimulated keratinocytes showed activation of three types of MAPKs; ERK, JNK, and p38 (Kim et al., 2011). It has been reported that p38, but neither ERK nor JNK is involved in TNF- α /IFN- γ -stimulated expression of CCL17 in HaCaT cells (Nakayama et al., 2004; Qi et al., 2011). However, we observed that galangin significantly inhibited the activation of ERK and p38 in HaCaT cells. This study provides evidence that the inhibitory effect of galangin on the expression of pro-inflammatory cytokines and CCL17 is mediated by blocking ERK, p38 MAPKs and NF- κ B pathways in HaCaT cells.

In conclusion, the present study demonstrates that topical application of galangin suppresses the development of AD-like skin lesions *in vivo* and *in vitro* models. Galangin inhibits the important cytokines for AD by the reduction of NF- κ B and MAPKs (ERK, p38) in HaCaT cells. We provide evidence that galangin could be a potential therapeutic candidate for AD. Our data imply that galangin may be natural remedies and effectively prevent atopic dermatitis, and can be used as a useful pharmacological agent or food supplement.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Transparency Document

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References

- Arbabi, S., Maier, R.V., 2002. Mitogen-activated protein kinases. *Crit. Care Med.* 30, S74–S79.
- Bae, Y., Lee, S., Kim, S.H., 2011. Chrysin suppresses mast cell-mediated allergic inflammation: involvement of calcium, caspase-1 and nuclear factor-kappaB. *Toxicol. Appl. Pharmacol.* 254, 56–64.
- Bieber, T., 2010. Atopic dermatitis. *Ann. Dermatol.* 22, 125–137.
- Borrelli, F., Maffia, P., Pinto, L., Ianaro, A., Russo, A., Capasso, F., Ialenti, A., 2002. Phytochemical compounds involved in the anti-inflammatory effect of propolis extract. *Fitoterapia* 73 (Suppl. 1), S53–S63.
- Brandt, E.B., Sivaprasad, U., 2011. Th2 cytokines and atopic dermatitis. *J. Clin. Cell. Immunol.* 2.
- Choi, E.J., Lee, S., Kim, H.H., Singh, T.S., Choi, J.K., Choi, H.G., Suh, W.M., Lee, S.H., Kim, S.H., 2011. Suppression of dust mite extract and 2,4-dinitrochlorobenzene-induced atopic dermatitis by the water extract of *Lindera obtusiloba*. *J. Ethnopharmacol.* 137, 802–807.
- Choi, J.K., Oh, H.M., Lee, S., Park, J.W., Khang, D., Lee, S.W., Lee, W.S., Rho, M.C., Kim, S.H., 2013. Oleonic acid acetate inhibits atopic dermatitis and allergic contact dermatitis in a murine model. *Toxicol. Appl. Pharmacol.* 269, 72–80.
- Cushnie, T.P., Lamb, A.J., 2006. Assessment of the antibacterial activity of galangin against 4-quinolone resistant strains of *Staphylococcus aureus*. *Phytomedicine* 13, 187–191.
- Dai, X., Sayama, K., Tohyama, M., Shirakata, Y., Hanakawa, Y., Tokumaru, S., Yang, L., Hirakawa, S., Hashimoto, K., 2011. Mite allergen is a danger signal for the skin via activation of inflammasome in keratinocytes. *J. Allergy Clin. Immunol.* 127 (806–814), e801–804.
- Dokmeci, E., Herrick, C.A., 2008. The immune system and atopic dermatitis. *Semin. Cutan. Med. Surg.* 27, 138–143.
- Gomez-Caravaca, A.M., Gomez-Romero, M., Arraez-Roman, D., Segura-Carretero, A., Fernandez-Gutierrez, A., 2006. Advances in the analysis of phenolic compounds in products derived from bees. *J. Pharm. Biomed. Anal.* 41, 1220–1234.
- Greaves, M.W., 2005. Antihistamines in dermatology. *Skin Pharmacol. Physiol.* 18, 220–229.
- Guttman-Yassky, E., Nogales, K.E., Krueger, J.G., 2011a. Contrasting pathogenesis of atopic dermatitis and psoriasis – part I: clinical and pathologic concepts. *J. Allergy Clin. Immunol.* 127, 1110–1118.
- Guttman-Yassky, E., Nogales, K.E., Krueger, J.G., 2011b. Contrasting pathogenesis of atopic dermatitis and psoriasis – part II: immune cell subsets and therapeutic concepts. *J. Allergy Clin. Immunol.* 127, 1420–1432.
- Gwak, J., Oh, J., Cho, M., Bae, S.K., Song, I.S., Liu, K.H., Jeong, Y., Kim, D.E., Chung, Y.H., Oh, S., 2011. Galangin suppresses the proliferation of beta-catenin response transcription-positive cancer cells by promoting adenomatous polyposis coli/Axin/glycogen synthase kinase-3beta-independent beta-catenin degradation. *Mol. Pharmacol.* 79, 1014–1022.
- Heo, M.Y., Sohn, S.J., Au, W.W., 2001. Anti-genotoxicity of galangin as a cancer chemopreventive agent candidate. *Mutat. Res.* 488, 135–150.
- Herman, S.M., Vender, R.B., 2003. Antihistamines in the treatment of dermatitis. *J. Cutan. Med. Surg.* 7, 467–473.
- Huh, J.E., Jung, I.T., Choi, J., Baek, Y.H., Lee, J.D., Park, D.S., Choi, D.Y., 2013. The natural flavonoid galangin inhibits osteoclastic bone destruction and osteoclastogenesis by suppressing NF-kappaB in collagen-induced arthritis and bone marrow-derived macrophages. *Eur. J. Pharmacol.* 698, 57–66.
- Jin, H., Kumar, L., Mathias, C., Zurakowski, D., Oettgen, H., Gorelik, L., Geha, R., 2009a. Toll-like receptor 2 is important for the T(H)1 response to cutaneous sensitization. *J. Allergy Clin. Immunol.* 123 (875–882), e871.
- Jin, H., Oyoshi, M.K., Le, Y., Bianchi, T., Koduru, S., Mathias, C.B., Kumar, L., Le Bras, S., Young, D., Collins, M., Grusby, M.J., Wenzel, J., Bieber, T., Boes, M., Silberstein, L.E., Oettgen, H.C., Geha, R.S., 2009b. IL-21R is essential for epicutaneous sensitization and allergic skin inflammation in humans and mice. *J. Clin. Invest.* 119, 47–60.
- Kawakami, T., Ando, T., Kimura, M., Wilson, B.S., Kawakami, Y., 2009. Mast cells in atopic dermatitis. *Curr. Opin. Immunol.* 21, 666–678.
- Khayyal, M.T., el-Ghazaly, M.A., el-Khatib, A.S., 1993. Mechanisms involved in the antiinflammatory effect of propolis extract. *Drugs. Exp. Clin. Res.* 19, 197–203.
- Kim, S.Y., Sohn, E.J., Kim, D.W., Jeong, H.J., Kim, M.J., Kang, H.W., Shin, M.J., Ahn, E.H., Kwon, S.W., Kim, Y.N., Kwon, H.J., Kim, T.Y., Lee, K.S., Park, J., Eum, W.S., Choi, S.Y., 2011. Transduced PEP-1-FK506BP ameliorates atopic dermatitis in NC/Nga mice. *J. Invest. Dermatol.* 131, 1477–1485.
- Kim, H.H., Bae, Y., Kim, S.H., 2013. Galangin attenuates mast cell-mediated allergic inflammation. *Food Chem. Toxicol.* 57, 209–216.
- Kim, H.H., Park, S.B., Lee, S., Kwon, T.K., Shin, T.Y., Park, P.H., Lee, S.H., Kim, S.H., 2014. Inhibitory effect of putranjivain A on allergic inflammation through suppression of mast cell activation. *Toxicol. Appl. Pharmacol.* 274, 455–461.
- Kwon, H.K., Lee, C.G., So, J.S., Chae, C.S., Hwang, J.S., Sahoo, A., Nam, J.H., Rhee, J.H., Hwang, K.C., Im, S.H., 2010. Generation of regulatory dendritic cells and CD4+Foxp3+ T cells by probiotics administration suppresses immune disorders. *Proc. Natl. Acad. Sci. USA* 107, 2159–2164.
- Kwon, D.J., Bae, Y.S., Ju, S.M., Goh, A.R., Youn, G.S., Choi, S.Y., Park, J., 2012. Casuarinin suppresses TARC/CCL17 and MDC/CCL22 production via blockade of NF-kappaB and STAT1 activation in HaCaT cells. *Biochem. Biophys. Res. Commun.* 417, 1254–1259.
- Lee, S., Yun, H.S., Kim, S.H., 2011. The comparative effects of mesoporous silica nanoparticles and colloidal silica on inflammation and apoptosis. *Biomaterials* 32, 9434–9443.
- Leung, D.Y., Bieber, T., 2003. Atopic dermatitis. *Lancet* 361, 151–160.
- Leung, D.Y.M., Boguniewicz, M., Howell, M.D., Nomura, I., Hamid, O.A., 2004. New insights into atopic dermatitis. *J. Clin. Invest.* 113, 651–657.
- Meyer, N., Zimmermann, M., Bugler, S., Bassin, C., Woehrl, S., Moritz, K., Rhyner, C., Indermitte, P., Schmid-Grendelmeier, P., Akdis, M., Menz, G., Akdis, C.A., 2010. IL-32 is expressed by human primary keratinocytes and modulates keratinocyte apoptosis in atopic dermatitis. *J. Allergy Clin. Immunol.* 125, 858–865.
- Nakayama, T., Hieshima, K., Nagakubo, D., Sato, E., Nakayama, M., Kawa, K., Yoshie, O., 2004. Selective induction of Th2-attracting chemokines CCL17 and CCL22 in human B cells by latent membrane protein 1 of Epstein-Barr virus. *J. Virol.* 78, 1665–1674.
- Neis, M.M., Peters, B., Dreuw, A., Wenzel, J., Bieber, T., Mauch, C., Krieg, T., Stanzel, S., Heinrich, P.C., Merk, H.F., Bosio, A., Baron, J.M., Hermanns, H.M., 2006. Enhanced expression levels of IL-31 correlate with IL-4 and IL-13 in atopic and allergic contact dermatitis. *J. Allergy Clin. Immunol.* 118, 930–937.
- Novak, N., Simon, D., 2011. Atopic dermatitis – from new pathophysiologic insights to individualized therapy. *Allergy* 66, 830–839.
- O'Mahony, L., Akdis, M., Akdis, C.A., 2011. Regulation of the immune response and inflammation by histamine and histamine receptors. *J. Allergy Clin. Immunol.* 128, 1153–1162.
- Oyoshi, M.K., He, R., Kumar, L., Yoon, J., Geha, R.S., 2009. Cellular and molecular mechanisms in atopic dermatitis. *Adv. Immunol.* 102, 135–226.
- Qi, X.F., Teng, Y.C., Yoon, Y.S., Kim, D.H., Cai, D.Q., Lee, K.J., 2011. Reactive oxygen species are involved in the IFN-gamma-stimulated production of Th2 chemokines in HaCaT keratinocytes. *J. Cell. Physiol.* 226, 58–65.
- Quemeneur, L., Michallet, M.C., Ferraro-Peyret, C., Saint-Mezard, P., Benetiere, J., Ducluzeau, M.T., Nicolas, J.F., Revillard, J.P., 2003. Immunosuppressive antimetabolites inhibit induction of contact hypersensitivity while lymphoablative drugs also prevent its expression. *Eur. J. Dermatol.* 13, 540–547.
- Saeki, H., Tamaki, K., 2006. Thymus and activation regulated chemokine (TARC)/CCL17 and skin diseases. *J. Dermatol. Sci.* 43, 75–84.
- Schmid-Grendelmeier, P., Simon, D., Simon, H.U., Akdis, C.A., Wuthrich, B., 2001. Epidemiology, clinical features, and immunology of the “intrinsic” (non-IgE-mediated) type of atopic dermatitis (constitutional dermatitis). *Allergy* 56, 841–849.
- Thomas, W.R., Smith, W.A., Hales, B.J., Mills, K.L., O'Brien, R.M., 2002. Characterization and immunobiology of house dust mite allergens. *Int. Arch. Allergy Immunol.* 129, 1–18.
- 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.
- Zimmermann, M., Koreck, A., Meyer, N., Basinski, T., Meiler, F., Simone, B., Woehrl, S., Moritz, K., Eiwegger, T., Schmid-Grendelmeier, P., Kemeny, L., Akdis, C.A., 2011. TNF-like weak inducer of apoptosis (TWEAK) and TNF-alpha cooperate in the induction of keratinocyte apoptosis. *J. Allergy Clin. Immunol.* 127, 200–207, e201–210.