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The inhibitory effect of naringenin on atopic dermatitis induced by DNFB in NC/Nga mice

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ABSTRACT

Aims: Atopic dermatitis (AD) is a chronic and relapsing inflammatory dermatitis characterized by pruritic and eczematous skin lesions. Here, we investigated the therapeutic effect of the fruit flavonoid naringenin on DNFB induced atopic dermatitis mice model.

Main methods: AD-like skin lesion was induced by repetitive skin contact with DNFB in NC/Nga mice and the effects of the fruit flavonoid naringenin were evaluated on the basis of histopathological findings of skin, ear swelling and cytokine production of CD4⁺T cells.

Key findings: Intraperitoneal injection of naringenin for one week after DNFB challenge significantly lowered ear swelling and improved back skin lesions. In addition, naringenin significantly suppressed production of interferon-gamma (IFN- γ) by activated CD4⁺ T cells and serum IgE level. Furthermore, naringenin reduced DNFB-induced infiltration of eosinophils, mast cells, CD4⁺ T cells, and CD8⁺ T cells in skin lesions.

Significance: Naringenin may suppress the development of AD-like skin lesions in DNFB-treated NC/Nga mice by reducing IFN- γ production of activated CD4⁺ T cells, serum IgE levels and infiltration of immune cells to skin lesion.

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Introduction

Atopic dermatitis (AD) is a common chronic inflammatory skin disease that has increased in industrialized countries over the last 30 years (Ring et al., 2001). AD is characterized by chronic and relapsing inflammatory dermatitis, and pruritic and eczematous skin lesions of the body and related to immunoglobulin E (IgE) production, and a type-1 and type-2 helper T cell (Th1/Th2) imbalance has been hypothesized as the etiology (Fedenko, 2003; Leung, 2000; Novak et al., 2003). Pathophysiology of AD has a complex mechanism that includes abnormal genetic and immunologic pathways and exposure to environmental or chemical agents (Fedenko, 2003; Leung, 2000; Novak et al., 2003), and thus, its pathogenesis has not been clearly defined. The most complaint of AD patients is itching. Repetitive scratching disrupted barrier function of skin that is increased susceptibility to allergic sensitization as well as microbial infections (Oyoshi et al., 2009). These processes increased the productions of cytokines, and chemokines, upregulated the expressions of adhesion molecules in the AD skin lesion and followed the infiltration of skin lesions by lymphocytes, mast cells, eosinophils, and neutrophils (Oyoshi et al., 2009).

AD is an inflammatory skin disease that has two distinct phases (Jin et al., 2009). In the acute phase, AD skin lesions are infiltrated with

CD4⁺T cells, which mainly secrete the Th2 cytokines IL-4, IL-5, and IL-13, while in the chronic phase, Th1 cells secrete IFN- γ . The chronic phase of AD shows Th1-type inflammation and delayed-type hypersensitivity (DTH) due to an IFN- γ response that induces tissue remodeling and dermal thickening via collagen accumulation (Galli et al., 2003; Lee et al., 2007).

NC/Nga mice have been used to an animal model of AD and these mice spontaneously develop AD-like eczematous skin lesions when maintained in air-uncontrolled conventional environment (Shiohara et al., 2004). This similarity between the clinical symptoms displayed by NC/Nga mice and AD in humans, models based on these mice are considered to provide important information regarding the understanding of AD (Shiohara et al., 2004). AD-like skin lesions can be derived under specific pathogen free (SPF) conditions by DNFB (2,4-dinitrofluorobenzene) sensitization (D.Y. Kim et al., 2009; T.H. Kim et al., 2009; Tomimori et al., 2005).

Naringenin (4',5,7-trihydroxy flavanone) is one of the most abundant flavonoids in peels of citrus fruits, grapes, and tomatoes (Francis et al., 1989; Lee et al., 2001; So et al., 1997). It is well known that naringenin has anti-oxidative, anti-atherogenic, and anti-cancer activities (Du et al., 2009; Galati et al., 2001; Knowles et al., 2000). It also exerts an anti-inflammatory effect by inhibiting nitric oxide and prostaglandin E₂ production (Takano-Ishikawa et al., 2006). Recently, it is reported that naringenin could suppress picrylchloride-induced contact hypersensitivity by inhibiting the proliferation and activation of T cells (Fang et al., 2010). In addition, it is known that naringenin suppresses the production

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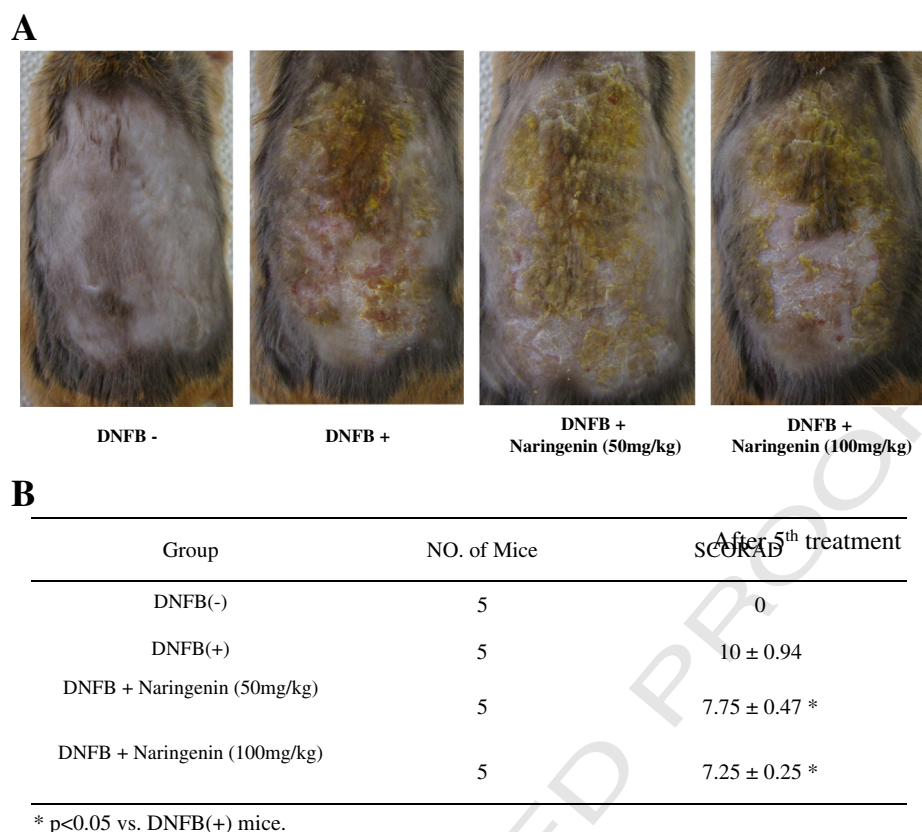


Fig. 1. Effect of naringenin treatment on AD-like skin lesions of DNFB treated NC/Nga mice. Atopic dermatitis-like lesions were induced by repeated painting of 0.15% DNFB (acetone:olive oil, 3:1) on dorsal skin of NC/Nga mouse once daily on days 1 and 4. The sensitized mice were further challenged with 0.2% DNFB on dorsal skin on days 7, 10, and 13. Control animals were treated with an identical volume of acetone/olive oil. Naringenin was injected i.p. daily from the day of first DNFB challenge (day 7). Representative photographs captured after treatment completion (fifth treatment) showing comparison of AD-like skin lesions (A). Five animals were allocated to each group. (B) The severity of AD was macroscopically assessed by SCORAD. The degree of each symptom was scored as 0 (absent), 1 (mild), 2 (moderate), and 3 (severe). The total score from the six symptoms of each mouse was taken as the score for that mouse. Data are presented as mean ± SEM of five determinations. *p < 0.05 vs. DNFB (+) mice.

of thymic stromal lymphopoietin in mast cells (Moon et al., 2011). However, there has been no study to investigate the effect of naringenin on AD-like skin lesions in NC/Nga mice.

Thus, in the present study, we investigated whether naringenin suppresses the formation of DNFB-induced AD-like skin lesions in NC/Nga mice and sought to reveal the mechanism involved.

Materials and methods

Animals

Six-week-old male NC/Nga mice were purchased from Japan SLC (Shizuoka, Japan) and maintained in SPF facilities. Animals were housed in an air-conditioned animal room (25 ± 1 °C; relative humidity 40 ± 5%) and fed a laboratory diet and distilled water. The experimental protocol used in this study complied with the guidelines issued by the ethical committee for animal welfare at Kyung Hee University [KHUASP (SE)-09-005], and all procedures were conducted in accordance with the guidelines issued by the U.S. National Institute of Health.

Naringenin treatment

The animals were divided into four groups: Control, DNFB, DNFB + Naringenin (50 mg/kg) and DNFB + Naringenin (100 mg/kg). Each group consisted of five mice. The Naringenin groups were treated with naringenin (Sigma Chemical, St. Louis, MO, USA) at 50 or 100 mg/kg i.p. daily from day 7 to day 13.

DNFB-induced dermatitis

The methods in this study have been described previously in D.Y. Kim et al. (2009), T.H. Kim et al. (2009). AD-like skin lesions were induced by the repeated application of 25 µL of 0.15% DNFB in acetone/olive oil (3:1) to the inner and outer surfaces of the ears and 100 µL of the same solution applied to shaved back skin once on days 1 and 4.

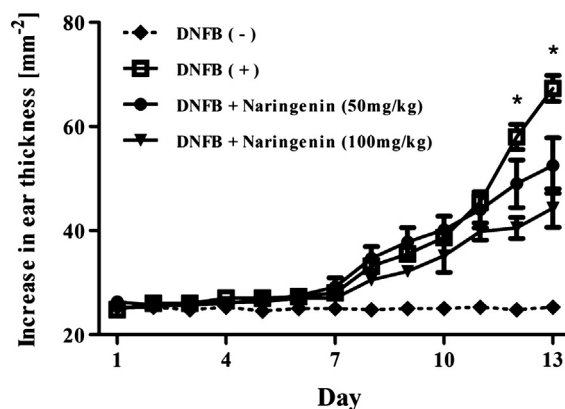


Fig. 2. Effect of naringenin treatment on ear swelling of DNFB treated NC/Nga mice. Ear swelling was measured daily using a thickness gauge. Naringenin was administered i.p. at concentrations of 100 or 200 ng/mouse. Five mice were allocated to each of the four groups: DNFB(-): acetone:olive oil (3:1) treated; DNFB(+): DNFB + PBS and ethanol 1% treated; DNFB + Naringenin (50 mg/kg); DNFB + Naringenin (100 mg/kg) treated. Ear thicknesses were measured with a thickness gauge every day. Values are mean ± SEM of five animals per group. *p < 0.05 (vs. the DNFB (+) group).

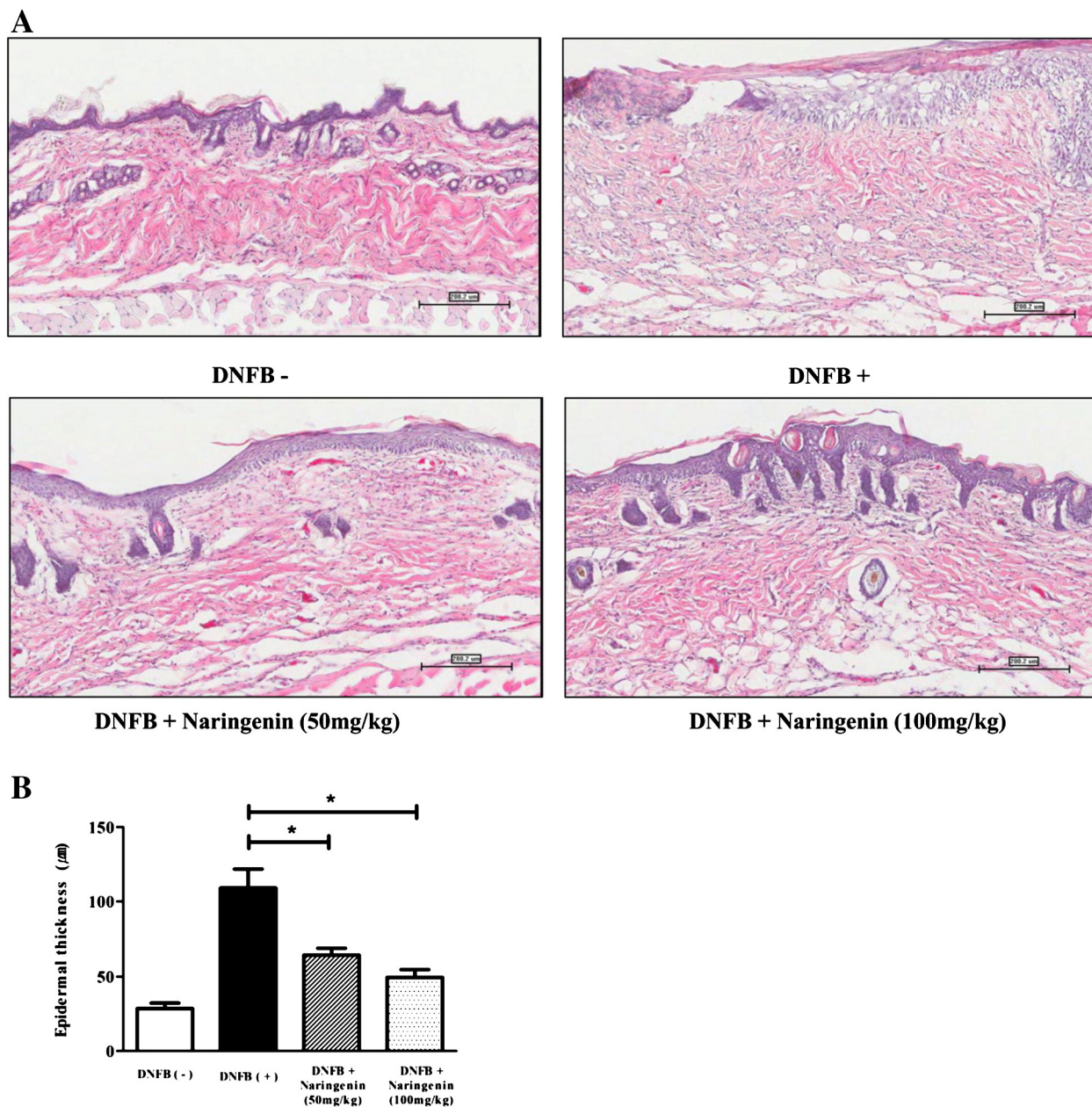


Fig. 3. Effect of naringenin on histological features of DNFB treated NC/Nga mice. NC/Nga mice were treated with 0.15% DNFB (acetone:olive oil, 3:1) on shaved dorsal skins on days 1 and 4, and further challenged with 0.2% DNFB on days 7, 10, and 13. Control animals were treated with an identical volume of acetone:olive oil. Naringenin was injected i.p. daily from the day of the first DNFB challenge (day 7). After each treatment, skin lesions were scored. After the fifth treatment, skin lesions were photographed and cryosections were prepared with hematoxylin and eosin (H&E) staining for histological analysis. A) H&E stained cryosections of dorsal skin samples to compare effect of naringenin treatment on skin inflammation. (B) Epidermal thickness (μm) (n = 24) in H&E stained sections was counted under a microscope. The number of cells is expressed as the mean ± SEM of five sections. *p < 0.05 vs. DNFB (+) mice; Bar = 200.2 μm.

On days 7, 10 and 13, sensitized mice were challenged by applying 0.2% DNFB to the back and ear skin surfaces. Control groups were treated with the same volumes of vehicle. Ear thicknesses were measured with a thickness gauge (Digimatic Indicator, Mitsutoyo, Tokyo, Japan). Macroscopic assessment of the severity of dermatitis was determined by a scoring method as described earlier (Hashimoto et al., 2006). Based on the severity of erythema/hemorrhage, edema, excoriation/erosion and dryness/scaling the degree of each sign was scored as 0 (none), 1 (mild), 2 (moderate) and 3 (severe). The total score (minimum 0, maximum 12) from the 5 symptoms of each mouse was taken as the score for that mouse. Assessment was done by an investigator who was blind about the grouping of the animals.

Serum IgE measurements

Total serum samples were prepared from blood collected 24 h after DNFB application on day 13, and the level of IgE concentrations in serum was detected by BD OptEIA™ Set Mouse IgE ELISA kits (BD Pharmingen, CA, USA).

Measurement of cytokine production in vitro

The methods in this study have been described previously in D.Y. Kim et al. (2009), T.H. Kim et al. (2009). T lymphocytes were isolated from the draining lymph nodes of mice, and CD4⁺ T cells were purified using a

BioMag separation column (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Isolated CD4⁺ T cells (1×10^6) were cultured in 24-well flat-bottom culture plates (Corning, Corning, NY, USA) in RPMI-1640 medium with 10% fetal bovine serum, 50 μ M β -mercaptoethanol, and 2 mM glutamine, then stimulated with 5 μ g/mL of immobilized anti-CD3 antibody (BD Pharmingen) and 2 μ g/mL of the soluble form of purified anti-CD28 antibody (BD Pharmingen). The production of IL-4 and IFN- γ after T cell activation was quantified by enzyme-linked immunosorbent assay (ELISA; Biolegend, CA, USA).

Histological analysis

Histological experiments were conducted as previously reported (D.Y. Kim et al., 2009; T.H. Kim et al., 2009). Skin tissues were removed 24 h after the final DNFB application and fixed with 4% paraformaldehyde in phosphate buffered saline (PBS, pH 7.4). Fixed tissues were washed in sucrose solution with several changes (10% sucrose in PBS for 4 h, 15% sucrose in PBS for 4 h, and 20% sucrose in PBS overnight) and frozen in OCT compound. Cryostat sections (8 μ m thick) were mounted on SuperFrost Plus slides (5116-20F, Muto. Pure Chemicals CO. LTD, Tokyo, Japan) and dried overnight at room temperature, before being fixed in ice-cold acetone for 10 min. Slides were rehydrated in PBS and blocked with 5% normal goat serum in PBS for 1 h at room temperature. After

washing with PBS, sections were incubated with primary antibody (rat anti-CD4 or anti-CD8 antibody; BD Pharmingen) and filaggrin overnight at room temperature. They were then washed with PBS and incubated with a fluorescein isothiocyanate (FITC)-labeled anti-rat antibody for 2 h at room temperature. Cell nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI; Molecular Probes, Eugene, OR, USA). Sections were mounted with glycerol mounting medium (Dako, Carpinteria, CA, USA) and images were captured using a confocal microscopy system (Olympus, Tokyo, Japan). Skin sections were also stained with hematoxylin and eosin for inflammatory cells. Mast cells were stained using a toluidine blue (TB). Acetone-fixed frozen slides were rehydrated and immersed in 0.1% TB (Sigma-Aldrich, St. Louis, MO, USA) in 70% ethanol (EtOH) for 3 min, followed by rinsing three times in water and dehydration in a graded series of EtOH (95–99% EtOH). Finally, the sections were cleared in xylene and cover-slipped in VECTASHIELD anti-fading solution (Vector Laboratories, Burlingame, CA, USA). Numbers of CD4⁺ T cells, CD8⁺ T cells, and mast cells were expressed as average total counts in five fields of 100 μ m² ($\times 400$).

Statistical analysis

Results are expressed as means $\pm$ SEMs. Statistical analysis was performed using one-way ANOVA. P-values of <0.05 were considered statistically significant.

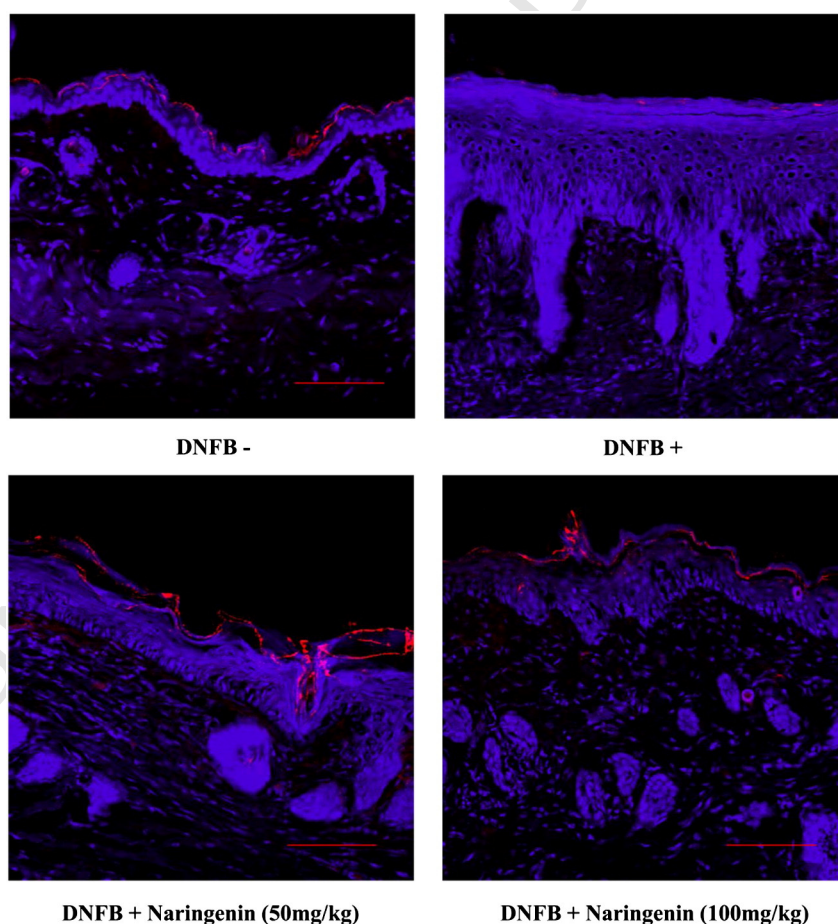


Fig. 4. Naringenin treatment recovered filaggrin expression in dorsal skin lesions. NC/Nga mouse were treated with 0.15% DNFB (acetone:olive oil, 3:1) on shaved back skins on days 1 and 4, and further challenged with 0.2% DNFB on days 7, 10, and 13. Control animals were treated with an identical volume of acetone/olive oil. Naringenin was injected i.p. daily from the day of first DNFB challenge (day 7). After final treatment with DNFB, immunofluorescent staining was done with the cryosections of back skin samples with fluorescein isothiocyanate. Representative picture showing expression of filaggrin after DNFB treatment, which was inhibited by naringenin dose-dependently (A). Data are mean $\pm$ SEM (n = 5). The dashed white lines indicate the approximate location of the epidermal basement membrane. The number of cells is expressed as the mean $\pm$ SEM of five sections. *p < 0.05 vs. DNFB (+) mice; Bar = 100 μ m.

Results

Naringenin reduced DNFB-induced AD-like skin symptoms

Naringenin has been known to exhibit anti-oxidative and anti-inflammatory effects (Du et al., 2009; Galati et al., 2001; Knowles et al., 2000; Takano-Ishikawa et al., 2006). We investigated whether naringenin reduced DNFB-induced AD-like symptoms.

The severity of symptoms in DNFB-treated mice was increased gradually with the number of DNFB challenges. Treatment of naringenin clearly reduced AD-like skin lesion development (Fig. 1). Ear swelling gradually increased from day 7. In order to investigate therapeutic effects of naringenin, we treated mice with naringenin daily from days 7 to 13. As result, ear swelling was ameliorated by naringenin treatment (Fig. 2). DNFB treatment can induce an epidermal hyperplasia in murine skin. As expected, repetitive DNFB treatments increased epidermal thickness (Fig. 3). In contrast, naringenin treatment clearly inhibited DNFB-induced epidermal hyperplasia (Fig. 3). The stratum corneum minimizes water loss from the body and protects against extreme environmental insults. Filaggrin (filament-aggregating protein) is a major structural protein in the stratum corneum of the epidermis.

Recently, it is reported that dust mite antigen and oxazolone treatment inhibit and altered the expression of filaggrin, an important contributor to epidermal barrier function (Yun et al., 2011). As expected, repetitive DNFB treatment disrupted filaggrin expression of epidermis, but the treatment of naringenin recovered filaggrin expression of epidermis (Fig. 4).

Naringenin IFN- γ production in activated CD4⁺ T cells

The cytokines produced by CD4⁺ T cells in draining lymph node determine their differentiation into Th1 and Th2 cells. CD4⁺ Th1 or Th2 cells seem to play a key role in the pathogenesis of AD. AD exhibits Th1 or Th2 dominant inflammation by the stage (Jin et al., 2009). Th1 and Th2 cells are classified by distinct cytokine production profiles (Spergel et al., 1999). Th1 cells mainly produce IFN- γ and IL-2, whereas Th2 cells produce IL-4, IL-5, and IL-13 (D.Y. Kim et al., 2009; T.H. Kim et al., 2009). In order to investigate whether naringenin modulates the Th1 and Th2 responses of CD4⁺ T cells in draining lymph nodes, we stimulated CD4⁺ T cells from the lymph nodes of non-treated or DNFB-treated or DNFB + Naringenin-treated mice. The secretion of IL-4 and IFN- γ in culture supernatants was measured by ELISA. As a result, administration of naringenin suppressed IFN- γ production after CD4⁺ T cell activation but not IL-4 (Fig. 5).

Naringenin reduces DNFB-induced serum IgE levels

Allergen recognized by IgE activates mast cell activation via its surface-localized Fc ϵ RI, which culminates in the release of preformed mediators, such as histamine and cytokines, including IL-4 and IL-13. Thus, it is conceivable that IgE contributes to the development of AD by mediating the activation of mast cells localized in the skin induced by allergens. Therefore, we investigated whether naringenin suppressed IgE in total serum. After the fifth DNFB treatment, serum samples were collected and total serum IgE levels were measured by ELISA. In mice topically treated with DNFB, total serum IgE levels were higher than in untreated controls (Fig. 6). However, treatment with 50 mg/kg or 100 mg/kg naringenin i.p. significantly reduced total serum IgE levels as compared with DNFB treated group (Fig. 6).

Naringenin treatment inhibits infiltration of inflammatory cells into DNFB-induced skin lesions

The infiltration of inflammatory cells in skin lesions is an important feature of AD. T lymphocytes in the skin lesions are the critical cellular mediators of the inflammatory dermal diseases (D.Y. Kim et al., 2009;

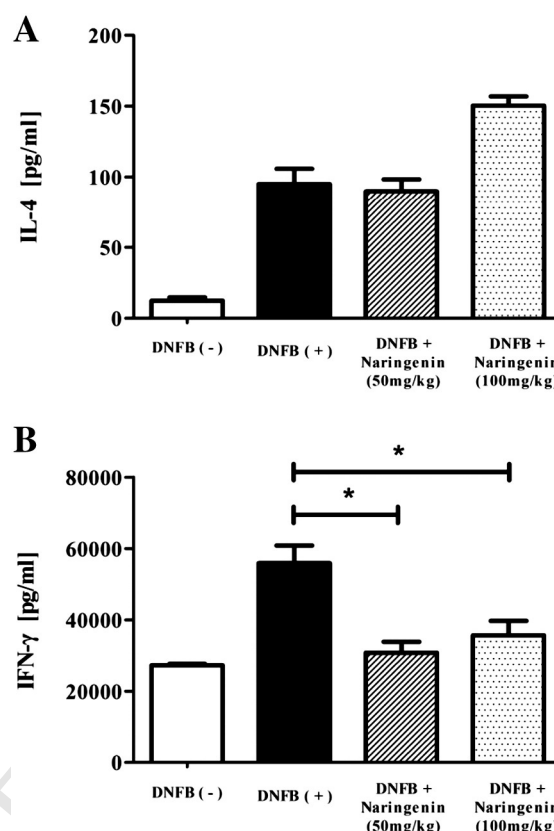


Fig. 5. Effect of naringenin on IL-4 (A) and IFN- γ (B) production by activated CD4⁺ T cells from lymph nodes of DNFB-treated NC/Nga mice. Lymph nodes were excised 24 h after final DNFB administration and CD4⁺ T cells were purified. The purified CD4⁺ T cells (1×10^6 cells/mL) were stimulated with plate-bound anti-CD3 antibody (2C11; 5 μ g/mL) plus a soluble form of purified anti-mouse CD28 antibody (2 μ g/mL) for 50 h. The production of IL-4 and IFN- γ after T cell activation was quantified by ELISA. Data are mean $\pm$ SEM (n = 5). *p < 0.05 vs. DNFB (+) mice.

T.H. Kim et al., 2009). Thus, we investigated whether infiltration of T lymphocytes in skin lesions was decreased by naringenin treatment.

Infiltration of CD4⁺ T and CD8⁺ T cell numbers in skin lesions was significantly inhibited by naringenin treatment (Fig. 7). Mast cells can be activated by allergen sensitized IgE, subsequently releasing granular mediators from granules, such as prostaglandin, leukotrienes and a large variety of cytokine (Bischoff, 2007; Kalesnikoff et al., 2001). Thus, we investigated the impact of naringenin on skin lesion infiltration of

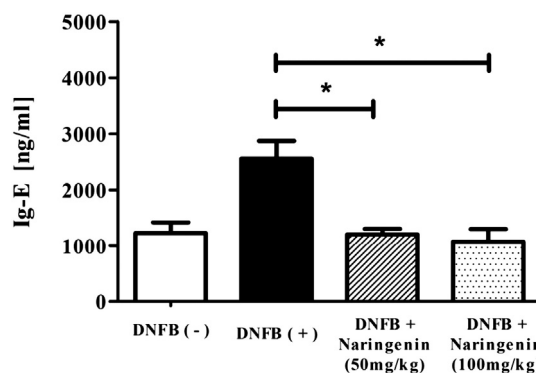
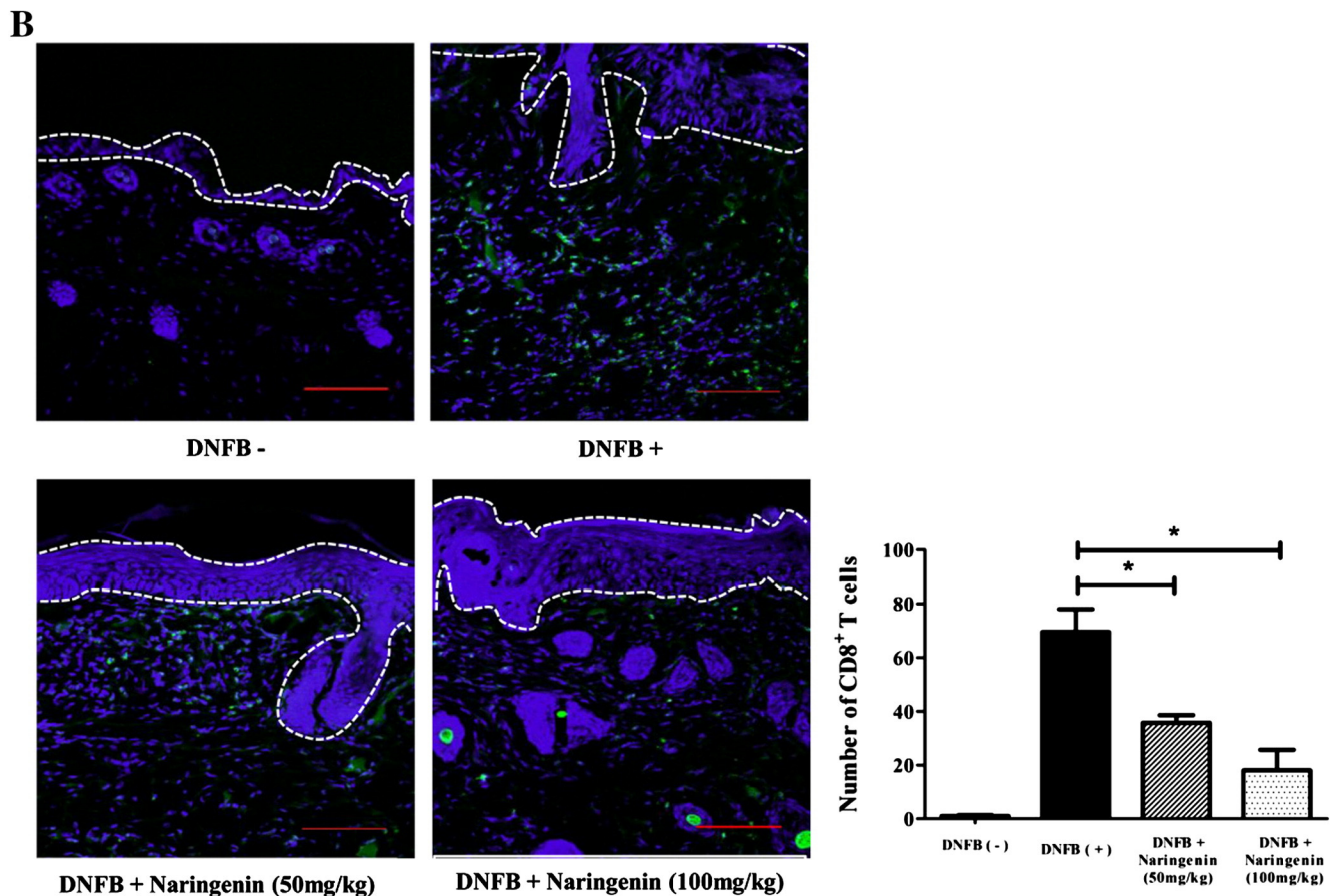
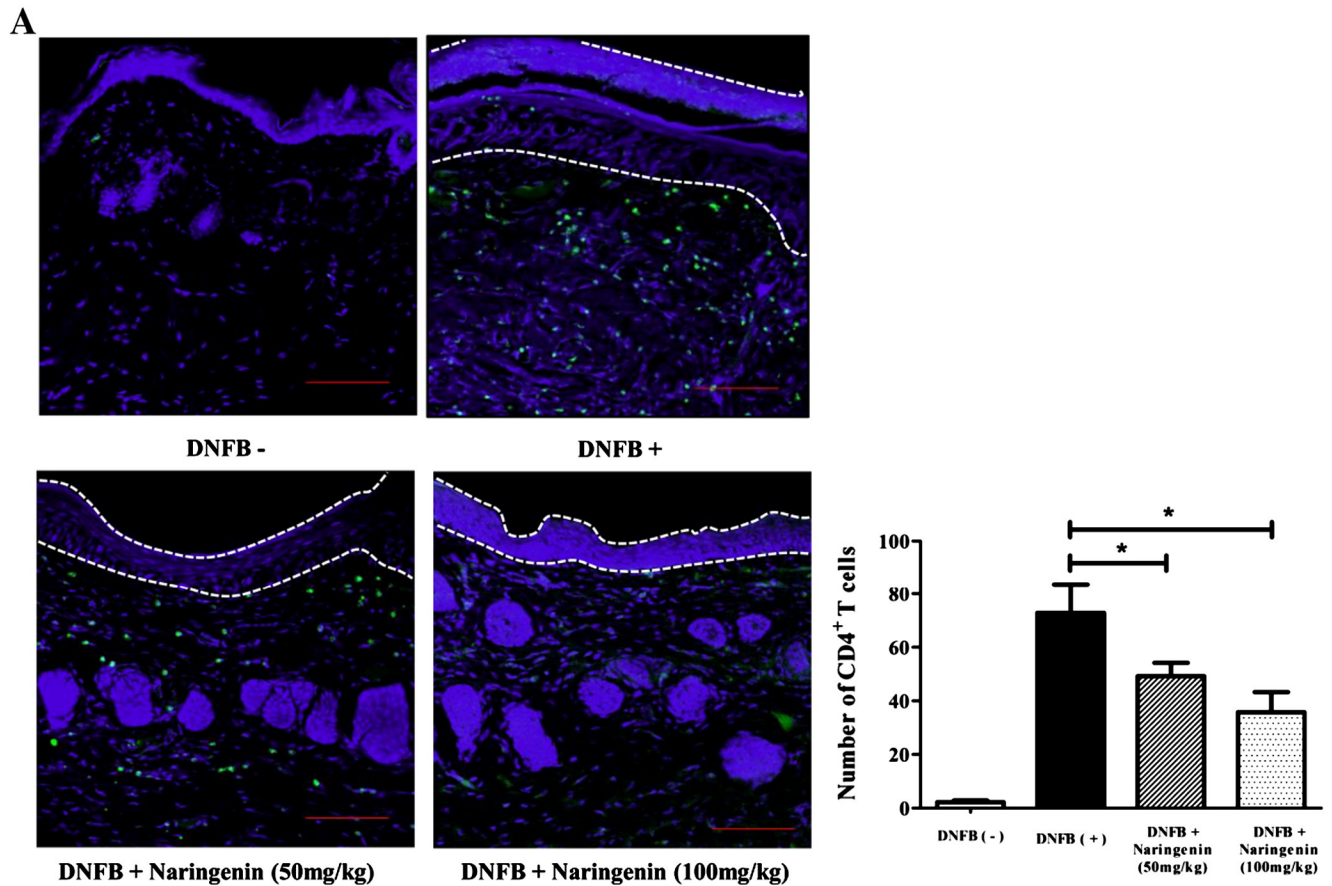


Fig. 6. Effect of naringenin on total IgE level of DNFB treated NC/Nga mice. IgE was quantified by ELISA in the serum obtained from DNFB-treated NC/Nga mice. Naringenin was injected i.p. daily from the day of the first DNFB challenge (day 7). Total IgE in the indicated groups was measured 24 h after the final administration. Data are mean $\pm$ SEM (n = 5). *p < 0.05 vs. DNFB (+) mice.



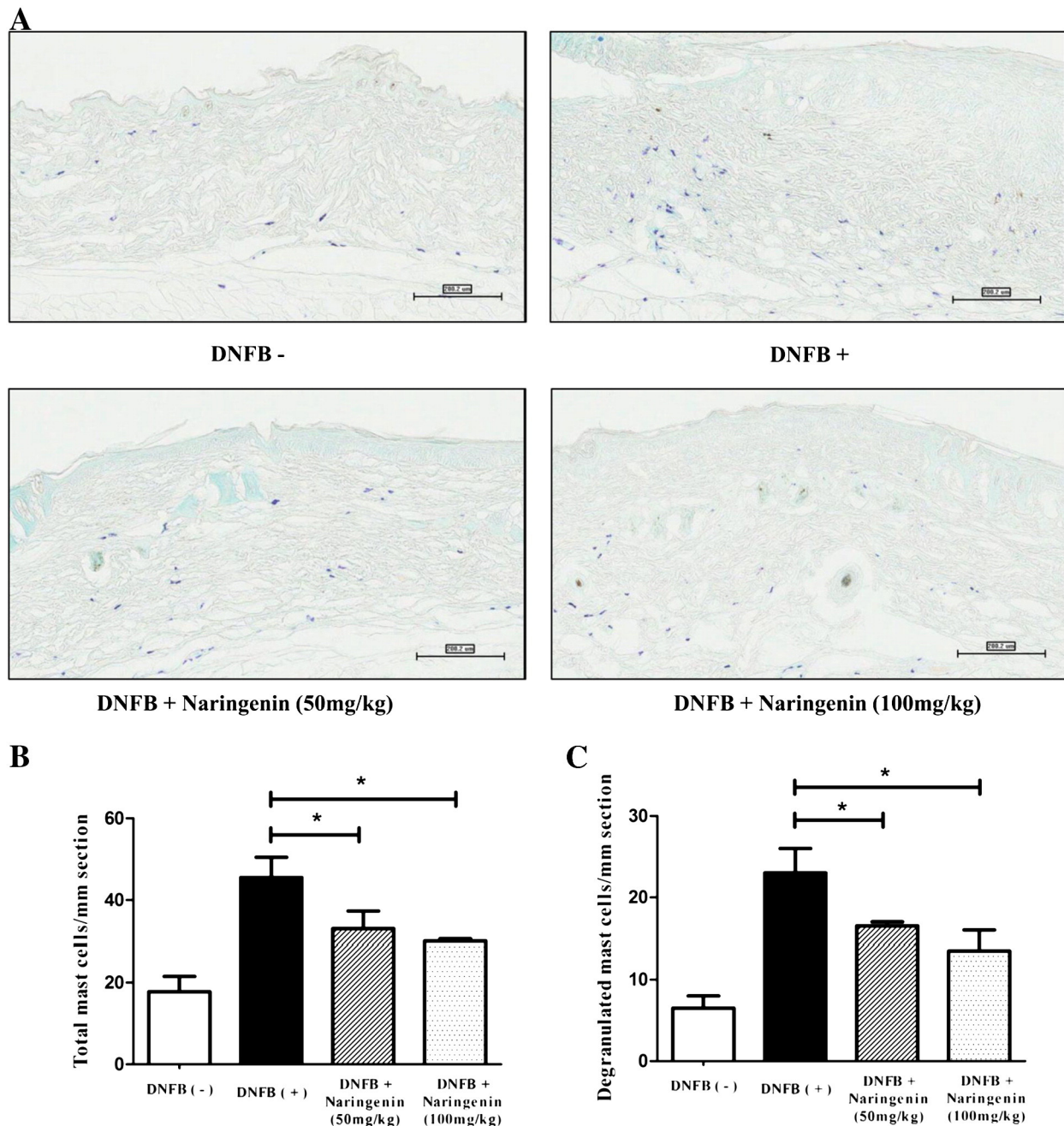


Fig. 8. Naringenin treatment reduces mast cell infiltration and degranulation in dorsal skin lesions. Mast cells (A) toluidine blue-stained sections. (B) Quantitative data for total mast cells and (C) degranulated mast cells ($n = 5$ sections each). Data are mean $\pm$ SEM ($n = 5$). * $p < 0.05$ vs. DNFB (+) mice; Bar = 200.2 μ m.

245 mast cells and degranulation using Toluidine Blue staining. The number
246 of infiltrated mast cells in skin lesion and degranulation markedly
247 inhibited after administration of 50 mg/kg or 100 mg/kg naringenin
248 treatment to mice (Fig. 8).

249 Discussion

250 Here we report that naringenin suppresses DNFB-induced atopic
251 dermatitis in NC/Nga mice. AD is a commonly encountered skin disease

and is characterized by erythema, edema, excoriation, and scaling. Un- 252
less there is a good effect of steroid to the treatment of AD, it cannot Q13
be treated in the long term due to its side effects. Therefore, researchers 254
have been trying to find new drugs, which are effective in AD. 255

It is well known that several effective agents involving flavonoid 256
compounds have been introduced for AD treatment (Gottlieb, 2005). 257
Naringenin is a well-known flavonoid in peels of citrus, grapes, and to- 258
matoes. Naringenin has a wide range of pharmacological properties, in- 259
cluding anticancer and anti-inflammatory effect (Francis et al., 1989; 260

Q3 **Fig. 7.** Effect of naringenin numbers of infiltrating CD4⁺ and CD8⁺ T cells in dorsal skin lesions of DNFB sensitized mice. NC/Nga mice were treated with 0.15% DNFB (acetone:olive oil, 3:1) on shaved back skins on days 1 and 4, and further challenged with 0.2% DNFB on days 7, 10, and 13. Control animals were treated with an identical volume of acetone/olive oil. Naringenin was injected i.p. daily from the day of first DNFB challenge (day 7). After the final treatment with DNFB, immunofluorescent staining was done with the cryosections of back skin samples with fluorescein isothiocyanate. Representative pictures showing activation of CD4⁺ T cells (A) and CD8⁺ T cells (B) after DNFB treatment, which was inhibited by naringenin dose-dependently. CD4⁺ T cells (C) and CD8⁺ T cells (D) were counted. The number of cells is expressed as the mean $\pm$ SEM of five sections. * $p < 0.05$ vs. DNFB (+) mice; Bar = 100 μ m.

Lee et al., 2001; So et al., 1997). In this study, we investigated the mechanisms by which naringenin improves AD using DNFB-induced AD in NC/Nga mice.

It is believed that T cells play critical roles in the pathogenesis of AD. A generalized Th2 immune response is linked to the condition of AD, but the skin lesion is a biphasic inflammation with an acute Th2 phase and a chronic Th1 phase (Galli et al., 2003; Lee et al., 2007). In clinical, the immune response of AD skin lesions is a mixture of Th1 and Th2 phases. Recently, it was reported that naringenin suppresses allergic asthma by inhibiting the Th2 type responses but not Th1 type responses (Iwamura et al., 2010). In contrast, in our present study, naringenin dose-dependently suppressed the production of IFN- γ by activated lymph node CD4⁺ T cells but not IL-4 (Fig. 5). It still remains unknown how the discrepancy occurred.

Elevated serum IgE levels have many effects on skin inflammation and IgE expression causes both acute and chronic phase atopic skin symptoms (Lee et al., 2006; Tanaka et al., 1994). Recently, it was known that naringenin treatment prevents the development of asthmatic airway inflammation via reduction of antigen induced Th2 cytokines, IL-4, IL-5, IL-13, but not serum IgE levels (Iwamura et al., 2010). On the other hand, Ying et al. reported that naringenin inhibits asthmatic airway inflammation through reduction of Th2 cytokines and serum IgE levels (Shi et al., 2009). In contrast, we found that naringenin treatment significantly reduced levels of total serum IgE in DNFB-treated mice but not IL-4 production of T cells (Figs. 5 & 6). Although this discrepancy is not clearly defined, this result could be explained by the efficacy of dose (Shi et al., 2009). Further studies are necessary to this topic.

Repetitive and long term contact to DNFB induces IFN- γ , a polarized Th1-type cytokine of activated T cells (Spergel et al., 1999). Chronic skin lesions are related with a dominant Th1-type response characterized by high levels of IFN- γ and IL-12. Additionally, in chronic AD patients, most of antigen reactive skin infiltrating T cell secretes IFN- γ (Tomimori et al., 2005). Recently, it was reported that naringenin inhibited the mRNA expressions of IL-2, IFN- γ , TNF- α in hapten-specific T cells and anti-CD3/anti-CD28 activated T cells. Similarly, we found that IFN- γ secretion by activated CD4⁺ T cells was increased in DNFB treatment groups, and this was clearly decreased by naringenin treatment (Fig. 5).

Leukocyte trafficking into the skin is mainly regulated by adhesion molecules and chemokines. Levels of chemokines and adhesion molecules, like intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) on endothelial cells, are increased in skin lesions and recruited by a variety of leukocytes, such as lymphocytes, mast cells, eosinophils, and neutrophils, to skin lesions (Homey et al., 2006; Kang et al., 2008; Mihara et al., 2004). Additionally, Th2 cells recruited into AD lesions by the interaction between CCL17 and CCR4 (Shibata et al., 2010). Akdis et al., reported histological findings have shown that the majority of skin-infiltrating T cells in active AD lesion are CD4⁺ T cells in the human AD (Akdis et al., 2003). It is reported that naringenin attenuates inflammatory cell recruitment via inhibition of chemokine production to the inflammatory sites in murine models of asthma, and diabetes (Shi et al., 2009). We also found that DNFB treatment increased the number of CD4⁺ and CD8⁺ T cells infiltrating skin lesions. In contrast, naringenin treatments reduced infiltrating CD4⁺ T and CD8⁺ T cell numbers after DNFB treatment (Fig. 7).

Mast cells are hematopoietically derived cells that reside long-term in barrier sites, such as skin and major effector cells in allergic disorders and during IgE-associated immune responses (Kitawaki et al., 2006). Allergen-IgE mediated activated mast cells secrete arachidonic acid metabolites, and various cytokines and chemokines by degranulation (Galli et al., 2005). The increase of infiltrated mast cells in the upper dermis is a critical feature of AD skin lesions (Matsuoka et al., 2003). Moon et al. reported that naringenin suppresses the production of thymic stromal lymphopoietin (TSLP) in mast cells (Moon et al., 2011). In our data, naringenin significantly reduced the number of mast cell infiltration and degranulation in DNFB-induced AD skin lesions (Fig. 8).

Filaggrin is a key protein that plays an important role in the formation of the cornified cell envelope, which is critical for an effective skin barrier (Howell et al., 2007). Recently, it is reported that dust mite antigen and oxazolone treatment inhibit and altered the expression of filaggrin (Yun et al., 2011). In addition, it is reported that several cytokines, IL-4, IL-13, IL-17, and IL-25, downregulate the expression of filaggrin (Gutowska-Owsiak et al., 2012; Pellerin et al., 2013). In this study, naringenin recovered filaggrin expression of epidermis in skin lesions (Fig. 4). However, we didn't define whether naringenin suppresses the expression of IL-13, IL-17, and IL-25 in this study. Thus, further studies are necessary at this point.

Conclusion

Naringenin reduces the development of the AD-like skin lesions on NC/Nga mice induced by repeated DNFB treatment by suppressing total serum IgE levels and the production of IFN- γ by activated CD4⁺ T cells, and by reducing lesion infiltration by CD4⁺ T, CD8⁺ T, and mast cells.

Conflict of interest statement

The authors have declared no conflict of interest.

Acknowledgments

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