



Omega-3 fatty acid-derived mediator, Resolvin E1, ameliorates 2,4-dinitrofluorobenzene-induced atopic dermatitis in NC/Nga mice

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ABSTRACT

Atopic dermatitis (AD) is a common inflammatory skin disease for which few effective treatments are available. Resolvin E1 (RvE1; 5S,12R,18R-trihydroxy-6Z,8E,10E,14Z,16E-eicosapentaenoic acid) is an endogenous lipid mediator derived from omega-3 fatty eicosapentaenoic acid, which is a potent inhibitor of inflammation. AD-like skin lesion was induced by repetitive skin contact with DNFB in NC/Nga mice and the effects of RvE1 were evaluated on the basis of histopathological findings of skin, ear swelling and cytokine production of CD4⁺ T cells. Intraperitoneal injection of RvE1 for one week after DNFB challenge significantly lowered ear swelling and improved back skin lesions. In addition, RvE1 significantly suppressed production of interferon-gamma (IFN- γ) and interleukin-4 (IL-4) by activated CD4⁺ T cells and serum IgE level. Furthermore, RvE1 reduced DNFB-induced infiltration of eosinophils, mast cells, CD4⁺ T cells, and CD8⁺ T cells in skin lesions. Therefore, RvE1 may suppress the development of AD-like skin lesions in DNFB-treated NC/Nga mice by reducing IL-4 and IFN- γ of activated CD4⁺ T cells and serum IgE levels and infiltration of immune cells to skin lesion.

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1. Introduction

Omega-3(ω -3) polyunsaturated fatty acids (n-3 PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are enriched in fish oils, are a safe and effective therapy in several human inflammatory disorders [1–6]. Resolvins are endogenous lipid mediators generated during the resolution phase of acute inflammation from n-3 PUFAs. Resolvin E1 (RvE1; 5S,12R,18R-trihydroxyeicosapentaenoic acid) is a member of the E series of resolvins, which is isolated from eicosapentaenoic acid (EPA) [7–10]. RvE1 markedly reduces dendritic cell migration, interleukin (IL)-12 production, neutrophil transendothelial migration, peritonitis, and dermal inflammation in complex disease models [9,11–13]. Anti-inflammatory mechanism of RvE1 protects against the development of colitis and blocks polymorphonuclear leukocyte (PMN) infiltration in periodontal disease in vivo [14,15].

The incidence of atopic dermatitis (AD) in children has increased in industrialized countries. AD is characterized by chronic and relapsing inflammatory dermatitis, immunological disturbance, and pruritic and eczematous skin lesions related to immunoglobulin E (IgE) production. A type-1 and type-2 helper T cell (Th1/Th2) imbalance has been hypothesized as the etiology. The pathophysiology of AD involves a complex mechanism that includes abnormal genetic and

immunologic pathways and exposure to environmental or chemical agents [16–18]. Because of the complexity, its pathogenesis has not been clearly defined.

Itching is the most complaint of AD patients. Repetitive scratching disrupts the barrier function of skin that increased the susceptibility to allergic sensitization as well as microbial infections. These processes increase the production of cytokines and chemokines, and up-regulates the expression of adhesion molecules in AD skin lesions, which is followed by infiltration of the skin lesions by lymphocytes, mast cells, eosinophils, and neutrophils.

AD is an inflammatory skin disease that can be considered to have two distinct phases [19]. During the acute phase, AD skin lesions are infiltrated with CD4⁺ T cells, which predominantly secrete the Th2 cytokines IL-4, IL-5, and IL-13, whereas in the chronic phase, Th1 cells secrete interferon-gamma (IFN- γ). The chronic phase of AD involves Th1-type inflammation and delayed-type hypersensitivity (DTH) due to an IFN- γ response that triggers tissue remodeling and dermal thickening via collagen accumulation [20,21].

NC/Nga mice are an established animal model of AD. The mice spontaneously develop AD-like eczematous skin lesions when maintained in an air-uncontrolled conventional environment [22]. Given the similarity between the clinical symptoms displayed by NC/Nga mice and AD in humans, findings from the model are readily translatable to human AD. AD-like skin lesions can be induced under specific pathogen free (SPF) conditions by 2,4-dinitrofluorobenzene (DNFB) [23–25].

The administration of RvE1 decreases airway eosinophil and lymphocyte recruitment, suppresses the production of the Th2 cytokine IL-13 and circulating ovalbumin-specific IgE, promotes the resolution

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of inflammatory airway responses by directly inhibiting the production of IL-23, IL-17, and IL-6 in the lungs of ovalbumin-sensitized Balb/c mice [26,27]. However, the effects of RvE1 on AD-like skin lesions in NC/Nga mice are unknown.

The present study investigated the influence of RvE1 on the suppression of the formation of DNFB-induced AD-like skin lesions in NC/Nga mice and the mechanism involved.

2. Materials and methods

2.1. Animals

Six-week-old male NC/Nga mice were obtained from Japan SLC (Shizuoka, Japan) and were maintained under SPF conditions. Animals were housed in an air-conditioned animal room ($25 \pm 1^\circ\text{C}$; relative humidity $40 \pm 5\%$) and were 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 United States National Institute of Health.

2.2. RvE1 treatment

The animals were randomly assigned to four groups ($n=5$ per group): Control, DNFB, DNFB + RvE1 (100 ng/mouse) and DNFB + RvE1 (200 ng/mouse). The RvE1 groups were treated with RvE1 (Cayman Chemical, Ann Arbor, MI, USA) at 100 or 200 ng/mouse via intraperitoneal (i.p.) injection daily from days 7 to 13.

2.3. DNFB-induced dermatitis

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. 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 [28–30]. 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 five 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.

2.4. Measurement of total and specific IgE in serum

Total serum samples were prepared from blood collected 24 h after DNFB application on day 13 and IgE concentrations in serum were quantified using BD OptEIA™ Set Mouse IgE ELISA kits (BD Pharmingen, San Diego, CA, USA) and mouse anti-DNP IgE ELISA kits (Alpha Diagnostic International, San Antonio, TX, USA).

2.5. Measurement of cytokine production in vitro

T lymphocytes were isolated from the draining lymph nodes of mice, and CD4^+ T cells were purified using a Biomag separation column

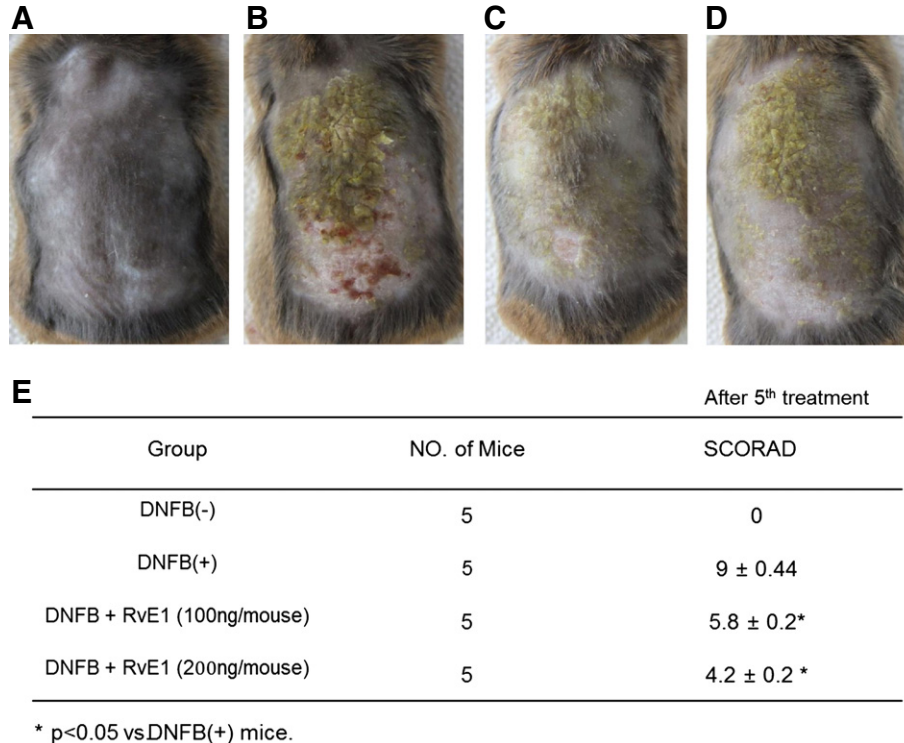


Fig. 1. Effect of RvE1 treatment on AD-like skin lesions of DNFB treated NC/Nga mice. Atopic dermatitis-like lesions were induced by repeated painting of 0.15% DNFB (acetone:olive oil, 3:1) on dorsal skin of NC/Nga mouse once daily on days 1 and 4. The sensitized mice were further challenged with 0.2% DNFB on dorsal skin on days 7, 10, and 13. Control animals were treated with an identical volume of acetone/olive oil. RvE1 was injected i.p. daily from the day of first DNFB challenge (day 7). (A) DNFB(-), (B) DNFB(+), (C) DNFB + RvE1 (100 ng/mouse), and (D) DNFB + RvE1 (200 ng/mouse) treated. Representative photographs captured after treatment completion (fifth treatment) showing comparison of AD-like skin lesions. Five animals were allocated to each group. (E) 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 $\pm$ SEM of five determinations. * $p < 0.05$ vs. DNFB(+) mice.

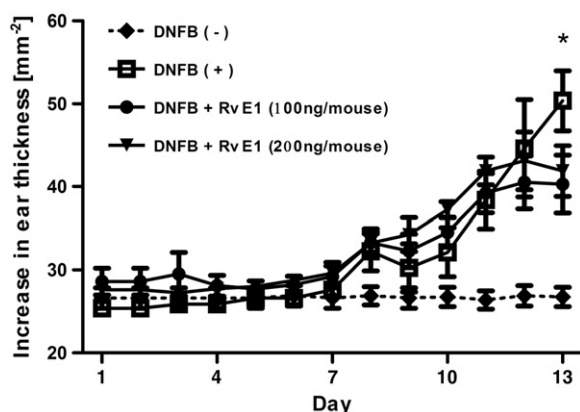


Fig. 2. Effect of RvE1 treatment on ear swelling of DNFB treated NC/Nga mice. Ear swelling was measured daily using a thickness gauge. RvE1 was administered i.p. at concentrations of 100 or 200 ng/mouse. Five mice were allocated to each of the four groups: DNFB(–): acetone:olive oil (3:1) treated; DNFB(+): DNFB + PBS and ethanol 1% treated; DNFB + Resolvin E1 (100 ng/mouse); DNFB + Resolvin E1 (200 ng/mouse) treated. Ear thicknesses were measured with a thickness gauge every day. Values are mean $\pm$ SEM of five animals per group. * $p < 0.05$ (vs. the DNFB(+) group).

(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).

2.6. Histological analysis

Skin tissues were removed 24 h after 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) 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 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 (X 400).

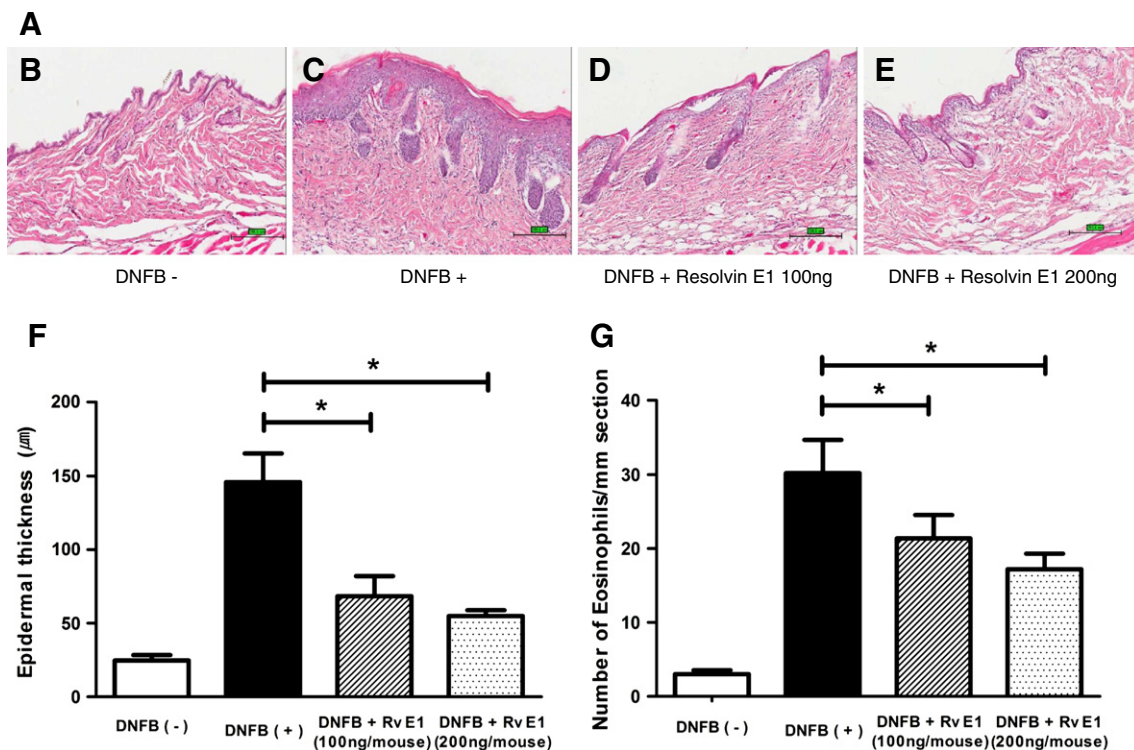


Fig. 3. Effect of RvE1 on histological features of DNFB treated NC/Nga mice. NC/Nga mice were treated with 0.15% DNFB (acetone:olive oil, 3:1) on shaved dorsal skins on days 1 and 4, and further challenged with 0.2% DNFB on days 7, 10, and 13. Control animals were treated with an identical volume of acetone:olive oil. RvE1 was injected i.p. daily from the day of 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 RvE1 treatment on skin inflammation. (B) DNFB(–), (C) DNFB(+), (D) DNFB + RvE1 (100 ng/mouse), and (E) DNFB + RvE1 (200 ng/mouse) treated; Bar = 200.6 μ m. (F) Epidermal thickness (μ m) ($n = 24$) and (G) eosinophils in H&E stained sections were counted under a microscope. The number of cells is expressed as the mean $\pm$ SEM of five sections. * $p < 0.05$ vs. DNFB(+) mice.

2.7. Statistical analysis

Results are expressed as mean $\pm$ SEM. The significances of changes were evaluated using the Student's *t*-test. Differences between experimental groups were evaluated using analysis of variance. *P* values <0.05 were considered significant.

3. Results

3.1. RvE1 suppresses DNFB-induced AD-like skin symptoms

RvE1 has been reported to exhibit anti-inflammatory effects [7,9]. We investigated the influence of RvE1 on the alleviation of DNFB-induced AD-like symptoms. It has been well-established that repetitive application of DNFB induces AD-like skin lesions in NC/Nga mice [25,31–33]. As expected, the severity of symptoms in DNFB-treated mice increased gradually with the number of DNFB challenges. I.p. treatment of RvE1 at 100 ng/mouse and 200 ng/mouse clearly reduced AD-like skin lesion development (Fig. 1). Ear swelling gradually increased from day 7. In order to investigate therapeutic effects of RvE1, we treated mice with RvE1 daily from days 7 to 13. Ear swelling was partially inhibited by RvE1 at 100 ng/mouse and 200 ng/mouse treatment (Fig. 2). DNFB treatment can induce an epidermal hyperplasia in murine skin. Repetitive DNFB treatments increased epidermal

thickness (Fig. 3). In contrast, RvE1 inhibited DNFB-induced epidermal hyperplasia in a dose-dependent manner (Fig. 3).

3.2. RvE1 suppresses IL-4 and IFN- γ production in activated CD4⁺ T cells

The cytokines produced by CD4⁺ T cells in the skin lesion determine their differentiation into Th1 and Th2 cells. AD exhibits Th1 or Th2 dominant inflammation dependent on stage [19]. Th1 and Th2 cells are characterized by distinct cytokine production profiles [34]. Th1 cells produce large amounts of IFN- γ and IL-2, whereas Th2 cells produce IL-4, IL-5, and IL-13 [24]. To investigate whether RvE1 regulates 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 RvE1 + DNFB or DNFB-treated mice with anti-CD3 and anti-CD28 antibodies for 60 h. The production of IL-4 and IFN- γ in culture supernatants was measured by ELISA. RvE1 suppressed IL-4 and IFN- γ production after CD4⁺ T cell activation in a dose-dependent manner (Fig. 4).

3.3. RvE1 reduces DNFB-induced serum IgE levels

AD is characterized by elevated total serum IgE levels, and IgE synthesis by B cells is probably modulated by the IL-4 secreted by Th2 cells [34]. IgE activates mast cells activation via its surface-localized Fc ϵ RI, which culminates in the release of preformed mediators, such

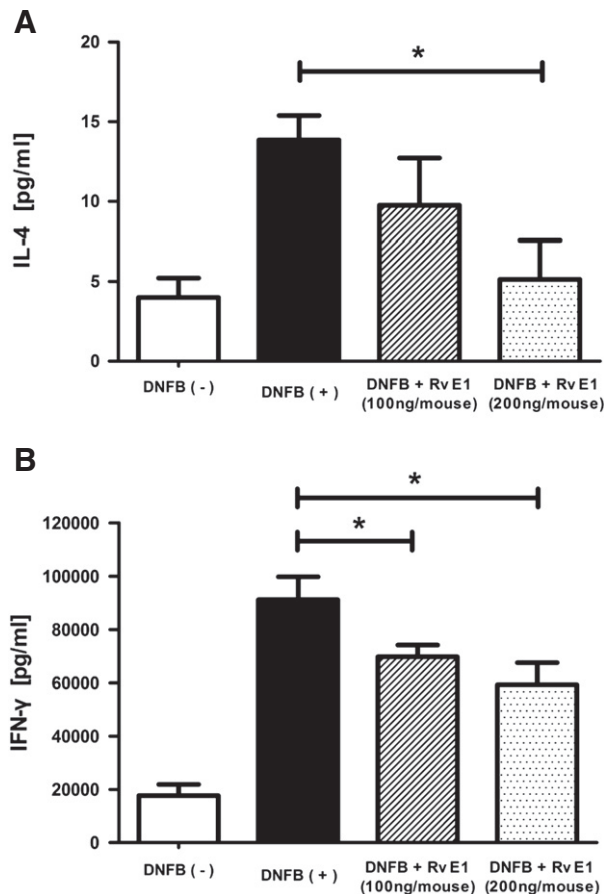


Fig. 4. Effect of RvE1 on IL-4(A) and IFN- γ (B) production by activated CD4⁺ T cells from lymph nodes of DNFB-treated NC/Nga mice. Lymph nodes were excised 24 h after final DNFB administration and CD4⁺ T cells were purified. The purified CD4⁺ T cells (1×10^6 cells/mL) were stimulated with plate-bound anti-CD3 antibody (2C11; 5 μ g/mL) plus a soluble form of purified anti-mouse CD28 antibody (2 μ g/mL) for 60 h. The production of IL-4 and IFN- γ after T cell activation was quantified by ELISA. DNFB(-): acetone:olive oil (3:1) treated; DNFB(+): DNFB + PBS and ethanol 1% treated; DNFB + RvE1 (100 ng/mouse); DNFB + RvE1 (200 ng/mouse) treated. Data are mean $\pm$ SEM (*n* = 5). **p* < 0.05 vs. DNFB(+) mice.

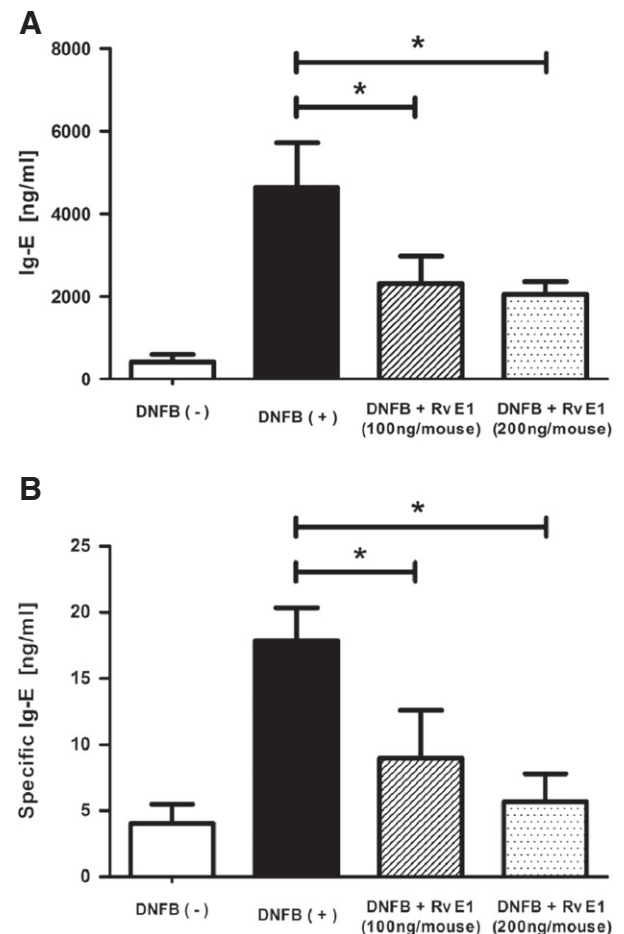


Fig. 5. Effect of RvE1 on total and DNP-specific serum IgE level of DNFB treated NC/Nga mice. IgE was quantified by ELISA in the serum obtained from DNFB-treated NC/Nga mice. RvE1 was injected i.p. daily from the day of first DNFB challenge (day 7). (A) Total IgE and (B) DNP-specific IgE in the indicated groups was measured 24 h after final administration. DNFB(-): acetone:olive oil (3:1) treated; DNFB(+): DNFB + PBS and ethanol 1% treated; DNFB + RvE1 (100 ng/mouse); DNFB + RvE1 (200 ng/mouse) treated. Data are mean $\pm$ SEM (*n* = 5). **p* < 0.05 vs. DNFB(+) mice.

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. Presently, IL-4 production by activated CD4⁺ T cells was reduced by RvE1 (Fig. 4A). Therefore, we investigated whether RvE1 suppressed IgE in serum. After the fifth DNFB treatment, serum samples were collected and total serum IgE and DNP-specific IgE levels were measured by ELISA. In mice topically treated with DNFB, total serum IgE and DNP-specific IgE levels were higher than in untreated controls (Fig. 5A, B). However, daily i.p. treatment with 100 ng/mouse or 200 ng/mouse RvE1 significantly reduced total serum IgE and DNP-specific IgE levels as compared with DNFB alone (Fig. 5A, B).

3.4. RvE1 reduces infiltration of inflammatory cells into DNFB-induced skin lesions

The infiltration of inflammatory cells in skin lesions is an important feature of AD. Cell types contained in infiltrates include lymphocytes

and mast cells. Eosinophils are immune cells that participate in defense against parasites and as mediators of allergic reactions. Eosinophilia is common in most patients with AD [35]. In this study, repeated DNFB treatments induced infiltration of eosinophils in AD-like skin lesions in the murine model (Fig. 3). However, infiltration of eosinophils in AD-like skin lesion was reduced by RvE1 treatments (Fig. 3). T lymphocytes in the skin lesions are the critical cellular mediators of the inflammatory dermal diseases [36]. We examined whether infiltration of T lymphocytes in skin lesions was decreased by RvE1. RvE1 reduced infiltration of CD4⁺ T and CD8⁺ T cell numbers in ear skin lesions in a dose-dependent manner (Fig. 6). Mast cells can be activated by allergen sensitized IgE via their cell surface high-affinity IgE receptor (FcεRI), subsequently releasing granular mediators, such as prostaglandin, leukotrienes and a large variety of cytokine [37,38]. Thus, we examined the effect of RvE1 on skin lesion infiltration and degranulation of mast cells using TB. Numbers of infiltrating mast cells in lesions and degranulation markedly decreased after administration of 100 ng or 200 ng RvE1 treatment to mice (Fig. 7).

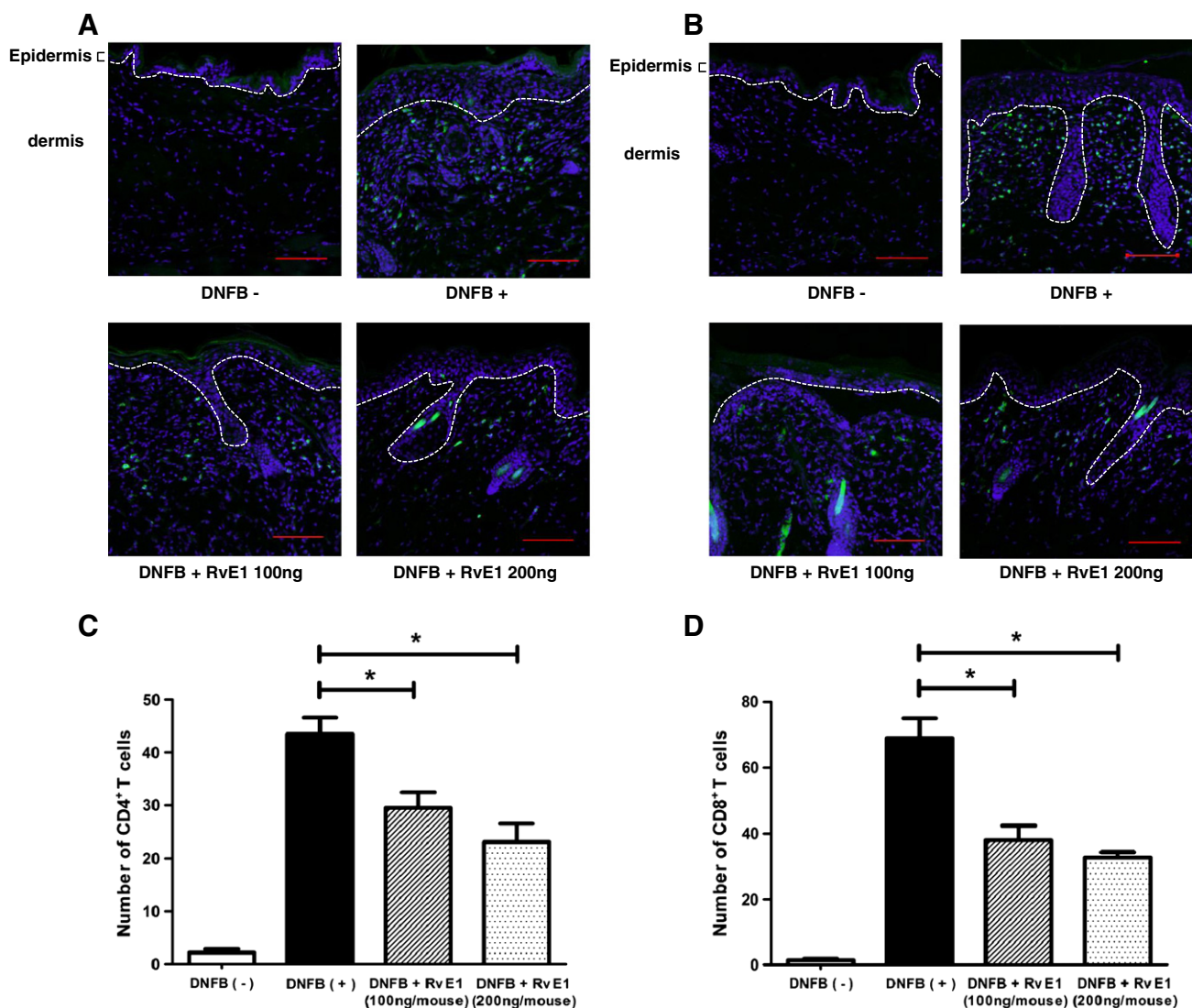


Fig. 6. Effect of RvE1 numbers of infiltrating CD4⁺ and CD8⁺ T cells in dorsal skin lesions of DNFB sensitized mice. NC/Nga mouse were treated with 0.15% DNFB (acetone:olive oil, 3:1) on shaved back skins on days 1 and 4, and further challenged with 0.2% DNFB on days 7, 10, and 13. Control animals were treated with an identical volume of acetone/olive oil. RvE1 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 pictures showing activation of CD4⁺ T cells (A) and CD8⁺ T cells (B) after DNFB treatment, which was inhibited by RvE1 dose-dependently. CD4⁺ T cells (C) and CD8⁺ T cells (D) were counted. The dashed white lines indicate the approximate location of the epidermal basement membrane. The number of cells is expressed as the mean ± SEM of five sections. **p* < 0.05 vs. DNFB(+) mice; Bar = 100 μm.

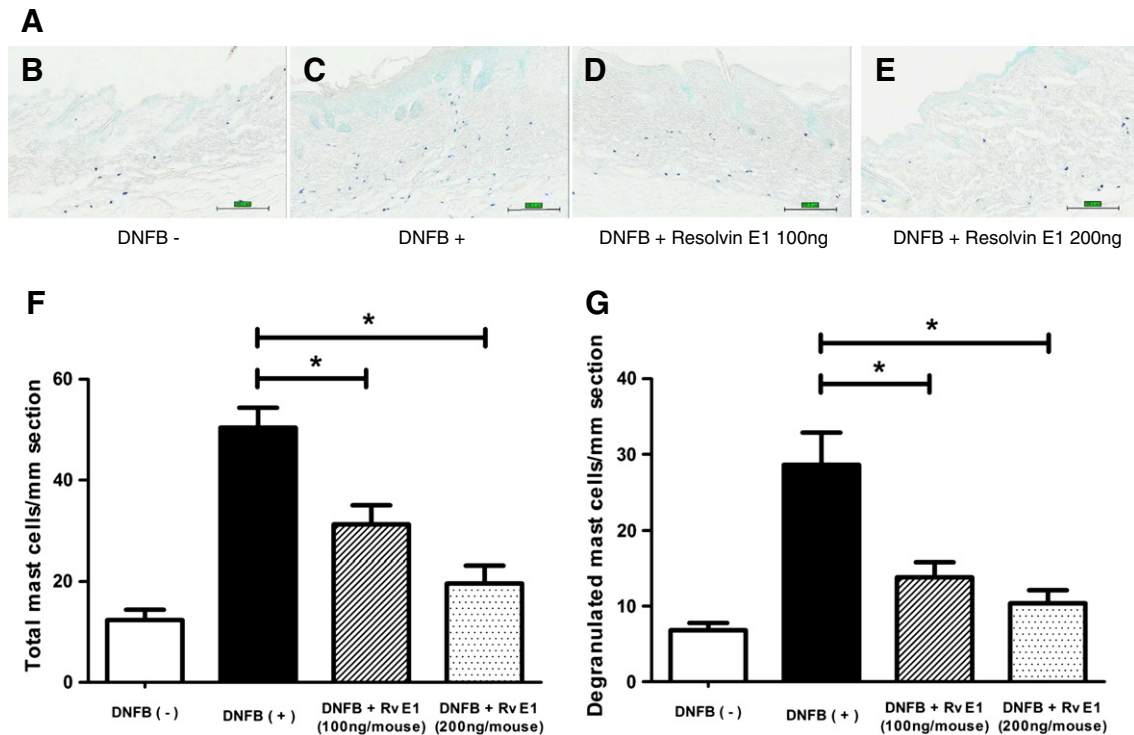


Fig. 7. RvE1 treatment reduces mast cell infiltration and degranulation in dorsal skin lesions. Mast cell (A) Toluidine blue-stained sections. (B) DNFB(-), (C) DNFB(+), (D) DNFB + RvE1 (100 ng/mouse), and (E) DNFB + RvE1 (200 ng/mouse) treated. (F) Quantitative data for total mast cells and (G) degranulated mast cells ($n=5$ sections each). Data are mean $\pm$ SEM ($n=5$). * $p<0.05$ vs. DNFB(+) mice; Bar = 200.6 μ m.

4. Discussion

AD is the most commonly encountered skin disease and is characterized by erythema, edema, excoriation, and scaling [39,40]. Although topical steroid therapy is very effective for the treatment of AD, it cannot be administered in the long-term due to its side effects. Thus, new types of therapy are required.

Lipid mediators are critical messengers in many physiological processes [3]. The pro-inflammatory effect of many prostaglandins derived from arachidonic acid is well established. However, there are also anti-inflammatory lipid mediators, such as resolvins and lipoxins that are derived from $n-3$ and $n-6$ PUFAs, control and resolve inflammation in a variety of experimental models of inflammatory disorders [9]. Recently, $n-3$ PUFA-derived lipid mediators such as RvE1 were shown to protect from experimental colitis in mouse models [14]. In this study, we examined the mechanisms by which RvE1 improves AD using DNFB-induced AD in NC/Nga mice.

T cells play critical roles in the pathogenesis of AD. Activated $CD4^{+}$ T cells induce the differentiation of Th1 cells to IFN- γ and Th2 cells that secrete IL-4, IL-5, and IL-13 [41]. In the present study, RvE1 dose-dependently suppressed the production of IFN- γ and IL-4 by activated lymph node $CD4^{+}$ T cells (Fig. 4). IL-4 (a Th2-type cytokine) induces class switching from IgM secreting B cells to IgE secreting B cells. Furthermore, elevated serum IgE levels have several effects on skin inflammation [42,43]. In particular, IgE expression causes both acute and chronic phase skin symptoms [42,43], and repeated painting with DNFB increases serum IgE levels in NC/Nga mice [19,23,24,44]. Recently, it was reported that RvE1 treatment prevents the development of asthmatic airway inflammation via reduction of antigen induced Th2 cytokines, IL-4, IL-5, IL-13, and serum IgE levels [26,27]. We also found that total serum IgE levels were significantly increased by repeated DNFB treatment in NC/Nga mice, as was IL-4 production by activated $CD4^{+}$ T cells obtained from these mice (Fig. 4). In contrast, RvE1 treatment significantly reduced levels of total

serum IgE in DNFB-treated mice and IL-4 production by activated $CD4^{+}$ T cells (Fig. 4).

Repeated exposure to DNFB induces IFN- γ a polarized Th1-type cytokine [45]. In addition, IFN- γ (a Th1-type cytokine) might play a role in determining the chronicity of AD lesions. In chronic AD patients, major antigen reactive skin infiltrating T cells secrete IFN- γ . Recently, it was reported that RvE1 improves 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced inflammatory bowel disease through inhibition of Th1 cytokine gene expressions in inflamed colons [14]. Similarly, we found that IFN- γ secretion by activated $CD4^{+}$ T cells was increased in DNFB treatment groups, and this was clearly decreased by RvE1 treatment (Fig. 4).

AD patients tend to scratch, which causes mechanical injury and induces the production of inflammatory cytokines and chemokines. Levels of chemokines and adhesion molecules, like intercellular adhesion molecule 1 and vascular adhesion molecule 1 on endothelial cells, are increased and recruited by a variety of leukocytes, such as lymphocytes, mast cells, eosinophils, and neutrophils, to skin lesions [46–49]. Furthermore, the interaction between CCL17 and CCR4 is regulated by the infiltration of Th2 cells into AD lesions by chemotaxis [50]. In human AD, histological findings have shown that the majority of skin-infiltrating T cells in active AD lesion are $CD4^{+}$ T cells [51]. RvE1 regulates cell surface adhesion molecules and chemokine receptors that are essential for leukocyte extravasation and recruitment to the inflammatory sites [52,53]. In addition, it is well-known that RvE1 attenuates polymorphonuclear cell recruitment to the inflammatory sites in murine models of acute inflammation including peritonitis, colitis, and dermal air pouch [14,15]. We also presently found that DNFB treatment increased the number of $CD4^{+}$ T cells infiltrating skin lesions. In contrast, RvE1 treatments reduced infiltrating $CD4^{+}$ T cells numbers after DNFB treatment (Fig. 6).

Recently, it was shown that allergen-primed $CD8^{+}$ T cells are required for the development of AD skin lesions in mice [54]. In the present study, RvE1 significantly reduced skin lesion infiltration by

CD8⁺ T cells (Fig. 6). However, in mouse and rat models, allergic inflammation was found to be dependent on CD4⁺ T cells, and CD8⁺ T cells were found to have a negative regulatory effect [49,55,56]. We do not know whether infiltrating CD8⁺ T cells provoke skin lesions. Further studies are required to clarify this point.

In particular, mast cells are pivotal effector cells in allergic disorders and during IgE-associated immune responses [57]. Upon activation, mast cells undergo degranulation and secrete arachidonic acid metabolites, and various cytokines and chemokines, which can all have immunoregulatory effects [58]. The infiltration of the upper dermis by mast cells is a critical feature of AD skin lesions [59]. Presently, RvE1 significantly reduced the number of mast cells infiltration and degranulation in DNFB-induced AD skin lesions (Fig. 7).

In summary, RvE1 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 and IL-4 by activated CD4⁺ T cells, and by reducing lesion infiltration by CD4⁺ T, CD8⁺ T, and mast cells.

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The authors have declared no conflict of interest.

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