



Berberine induces anti-atopic dermatitis effects through the downregulation of cutaneous EIF3F and MALT1 in NC/Nga mice with atopy-like dermatitis

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ABSTRACT

Atopic dermatitis (AD) is a chronic inflammatory skin disease with severe pruritus. Berberine, a naturally occurring isoquinoline alkaloid, has anti-inflammatory effects. This study investigated the effects and molecular mechanisms of berberine on AD-like symptoms in mice. In this study, NC/Nga mice with atopy-like dermatitis (dermatitis mice), fibroblast and mast cells were used. In dermatitis mice, intermittent oral administrations of berberine 3 times a week for 12 days inhibited skin symptom, itching, cutaneous infiltration of eosinophils and mast cells, and the expression of cutaneous eotaxin, macrophage migration inhibitory factor (MIF) and IL-4. Berberine also attenuated IL-4/MIF-induced eotaxin in fibroblasts and allergen-induced MIF and IL-4 in mast cells. In mast cells, the GeneChip® microarray showed that antigen increased the expression of EIF3F and MALT1, inhibited by berberine. The siRNAs for them inhibited the expression of MIF and IL-4 in antigen-stimulated mast cells. These results suggest that berberine improves AD-like symptoms through the inhibition of the eotaxin and pro-inflammatory cytokine expression and the related inflammatory cell recruitment. It is also suggested that the downregulation of EIF3F and MALT1 by berberine is involved in suppressing the cytokine expression. Taken together, berberine or berberine-containing crude drugs are expected to contribute to the improvement of AD symptoms.

1. Introduction

AD is one of the most common chronic inflammatory skin diseases. It is characterized by the infiltration of inflammatory cells, extensive pruritus and a clinical course of symptomatic flares and remissions [1]. The pathogenesis of the disease is now well understood, and important factors involved in the process include genetic factors, skin barrier dysfunction and immune dysregulation [2]. However, effective long-term treatment is sometimes difficult due to the chronic, relapsing nature of this disease, thus, there is a need to find better therapeutic options with minimal side effects that are well tolerated over the variable course of this disease. Currently, many patients visit physicians seeking

alternative treatments.

Berberine (Fig. 1A) is a naturally occurring isoquinoline alkaloid and is a major component of traditional Kampo medicine Orenge dokuto (OGT; Huang-Lian-Jie-Du-Tang in Chinese), which consists of four medicinal plants (*Coptidis rhizome*, *Phellodendri cortex*, *Scutellariae radix* and *Gardeniae fructus*) [3]. OGT is used for the treatment of AD [4,5]. Berberine has been reported to show anti-inflammatory and anti-oxidant effects [6–9]. Berberine suppresses inflammation through complex mechanisms that involve MAPKs, which are involved in regulating inflammatory mediators such as ERK, JNK, and p38 protein kinases at both transcriptional and translational levels [10]. In macrophages, it suppressed inflammatory response through AMP-activated protein

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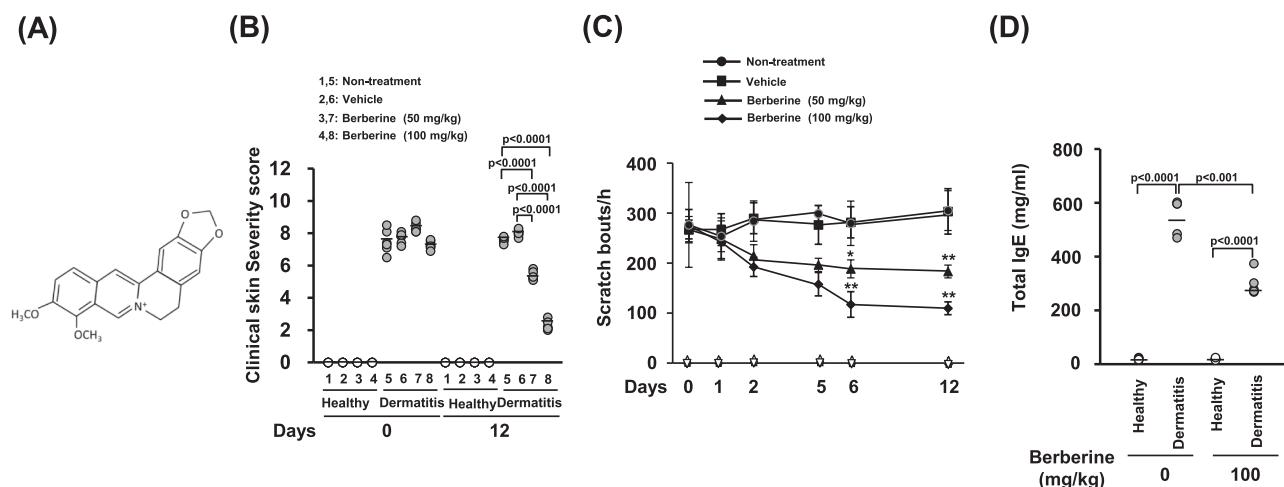


Fig. 1. The effects of berberine on atopic dermatitis-like symptoms in NC/Nga mice. In this study, healthy NC/Nga mice grown under specific pathogen free condition and dermatitis NC/Nga mice grown under conventional condition with mites were used. (A) Structure of berberine. The clinical skin severity scores (B) and scratching behaviour (C) of the NC/Nga mice after the oral administration of vehicle (tap water) or berberine (50 and 100 mg/kg) 3 times a week for 12 days. (D) The relationship between berberine (100 mg/kg) treatment and the serum total IgE levels on day 12. Each value represents the mean $\pm$ SD ($n = 5$). P value shows statistical significance. The data shown are representative of three independent experiments.

kinase activation, demonstrating its anti-inflammatory potency [7]. Furthermore, berberine has been reported to block the induction of oedema by 12-*O*-tetradecanoylphorbol-13-acetate on the ears of mice [11] and to inhibit PGE₂ level and cyclooxygenase-2 transcriptional activity through the regulation of activator protein-1 transcription factor [8,12]. Berberine has been reported to inhibit mast cell degranulation in a rat model of allergic contact dermatitis [13], inhibit the expression of MMP-9 and IL-6 in normal human keratinocytes [14], and to induce an anti-inflammatory response in mice with oxazolone-induced contact dermatitis [15]. However, whether berberine improves symptoms of AD, such as skin inflammation and itching, has remained unclear. Thus, in the present experiments, we investigated the efficacy of berberine against symptoms of AD as well as the molecular inhibitory mechanisms of berberine in a mouse model of AD.

2. Materials and methods

2.1. Materials

Berberine was obtained from Sigma-Aldrich (St. Louis, MO, USA). Berberine was used at doses *in vivo*, which does not affect the body weight and spleen weight. *In vitro*, the concentrations used showed no cytotoxicity based on our preliminary experiments.

In *in vivo* experiments, berberine or the vehicle (tap water) was orally administered 3 times a week for 12 days by gavage (oral zoned needle). Recombinant mouse Interleukin (IL)-4 was purchased from R&D systems (Minneapolis, MN, USA). Recombinant MIF was obtained from ProSpec (Newark, NJ, USA). In *in vitro* experiments, berberine and these cytokines were dissolved in culture medium.

2.2. Animals

Male NC/Nga mice (eight weeks old at the start of the experiments: Japan SLC, Shizuoka, Japan) were used for the study. AD-like lesion was induced by parasitizing mites according to previously described method [16,17]. Mice were housed in a room under controlled temperature (21–23 °C) and humidity (45%–65%) conditions with 12 h light (lights on from 07:00 to 19:00p.m.). Food and water were provided *ad libitum*. This study was carried out in strict accordance with the recommendations in the Institute for animal experiments at University of Toyama. The study protocol was approved by the ethics committee, and the animal experiments using mice were conducted according to the guidelines

set by the University of Toyama.

All experiments using mice were conducted under the blind condition.

2.3. Determining the clinical skin severity score

The clinical skin severity score was evaluated as in our previous study [18]. In brief, the clinical severity for AD-like lesions was evaluated as the sum of the individual scores, graded as 0 (none), 1 (mild), 2 (moderate), and 3 (severe) for each of five signs and symptoms (itch, erythema, edema, excoriation/erosion and scaling/dryness).

2.4. Behavioral evaluation

The behavioral analysis of mice was performed as described previously [19]. In brief, the animals were placed individually in an acrylic cage composed of 4 equal-sized cells (13 $\times$ 9 $\times$ 40 cm) for at least 1 h for acclimation. Their behaviors were then videotaped for 1 h, with personnel kept out of the observation room. Spontaneous scratching toward any rostral regions of the body by the hind paws was counted. As mice make several rapid scratch movements for periods of about 1 s each, a series of these movements was counted as one bout of scratching.

2.5. Determination of IgE

Blood (20 μ l) was collected from the heart of each mouse using a capillary glass tube under ether anesthesia, and centrifuged at 2,236 $\times$ g for 20 min at 4 °C. The serum was stored at –80 °C until the analyses. The total IgE in the serum was detected using an enzyme-linked immunosorbent assay kit (abcam Ltd., Cambridge, UK).

2.6. Histological analyses

At day 0 or day 12 after the treatment with berberine or the vehicle, under anesthesia with the mixed solution (0.15 ml/30 g), which contained medetomidine (0.3 mg/0.3 ml/kg Domitol; Meiji Seika Pharma Co., Ltd., Tokyo, Japan), midazolam (4 mg/0.8 ml/kg Dormicum; Astellas Pharma Inc., Tokyo, Japan) and butorphanol (5 mg/ml/kg Vetorphale; Meiji Seika Pharma Co., Ltd.), the mice were transcardially perfused with phosphate-buffered saline (PBS) followed by 10% formalin in neutral buffer solution (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The skin of the rostral back of the mouse was

isolated and post-fixed with 10% formalin in neutral buffer solution (Wako Pure Chemical Industries, Ltd.). The skin was embedded in paraffin and cut with a microtome into 2- μ m-thick sections. After deparaffinization, the sections were stained with hematoxylin & eosin (H&E) for the detection of eosinophils or toluidine blue for mast cells. Cells were counted at a magnification of $\times 400$ and expressed as the total number of cells in 5 fields (5 sections per mouse, 5 mice per group).

2.7. Cell culture and stimulation

Murine dermal fibroblasts were obtained from mice. Dermal fibroblasts were isolated by dissecting the dermal layer; these skin specimens were then floated in PBS containing 0.25% dispase (Life Technologies, Carlsbad, CA, USA) at 4 °C overnight. The fibroblasts were cultured and maintained in Dulbecco's modified Eagle's medium (DMEM; Nakarai, Kyoto, Japan) containing 10% fetal bovine serum (FBS, Summit Biotechnology, Ft. Collins, CO, USA), penicillin (100 units/ml) and streptomycin (100 μ g/ml) at 37 °C in a humidified atmosphere of 5% CO₂ in air. Cells were used between passages 2 and 8. The cells were cultured with IL-4 (1 ng/ml), MIF (100 ng/ml), and berberine (10 μ M) alone or in combination for 6 or 24 h. The concentration of cytokines in stimulation experiments was decided based on their basal circulating level according to our previous study [20]. MIF basal circulating levels are 1000-fold higher than other cytokines. In the relevant experiments, IL-4 (1 ng/ml), or MIF (100 ng/ml) was added.

To evaluate the effect of berberine on the antigen-mediated degranulation in mast cells, we used the evaluation system using DNP and the DNP-specific IgE. Briefly, the MC/9 murine mast cells (American Type Culture Collection, Manassas, VA, USA) were maintained by passaging in DMEM (Nakarai) supplemented with 5×10^{-5} M 2-mercaptoethanol (Sigma), 10% Rat T-STIM (Becton Dickinson, Heidelberg, Germany) and 10% FBS (Summit Biotechnology). The cells were then cultured with 500 ng/ml monoclonal anti-2,4-dinitrophenol-IgE (anti IgE-DNP; Sigma) in DMEM for 24 h. After washing twice with the culture medium, MC/9 cells were incubated with DNP-bovine serum albumin (BSA) (LSL, Tokyo, Japan, 100 ng/ml) and berberine (10 μ M) either alone or in combination for 6 or 24 h.

The specific siRNAs that were used as a control, *eukaryotic translation initiation factor 3 subunit F (EIF3F)* and *mucosa-associated lymphoid tissue protein 1 (MALT1)*, were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). These specific siRNAs were transfected at a concentration of 1 μ M using Lipofectamin RNAi MAX reagent (Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. An MTT assay was performed after 48 h in order to assess cell viability (data not shown). At 48 h after transfection, the cells were stimulated with DNP-BSA (100 ng/ml) for 24 h and then harvested for protein isolation.

2.8. Reverse transcription and real-time polymerase chain reaction (PCR) analyses

Total RNA was extracted from each mouse skin or cultured cells using the Nucleo Spin Extract II (Takara Bio, Shiga, Japan). Real-time quantitative PCR (qPCR) assay was performed on a real-time PCR system (Mx3005P, Stratagene, Tokyo, Japan) using SYBR PreMix ExTaq (Takara Bio, Shiga, Japan) or Premix ExTaq (for the use of TaqMan probes; Takara Bio) in accordance with the manufacturers' protocols. Reverse transcriptase reaction (Omniscript Reverse Transcriptase, Qiagen) was carried out with DNase-treated total RNA using oligo primers; *MIF*, *IL-4*, *IL-5*, *interferon (IFN)- γ* , *MALT1* and *EIF3F* (Bio-Rad, Hercules, CA, USA). The cycling conditions were 10 min at 95 °C, and then 40 cycles of 30 sec at 95 °C, 1 min at 60 °C, and 1 min at 72 °C. Each mRNA expression level was normalized to the mRNA expression level of glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*).

2.9. Western blot analyses

Frozen skin tissue or cells were homogenized in T-PER tissue extraction reagent (Pierce, Rockford, IL) containing protease inhibitor mixture and centrifuged at 12,000 rpm for 10 min at 4 °C. The protein content in the supernatants was measured using a Bio-Rad protein assay kit (Bio-Rad, Hercules, CA, USA). The protein lysates were separated using SDS-PAGE (5%-20% acrylamide gradients; ATTO Corporation, Tokyo, Japan) and transferred to nitrocellulose membranes using an Immobilon-P transfer membrane (Milli-pore Corp, Bedford, MA, USA). Nitrocellulose membranes were blocked with 5% skim milk in TBST and blotted with the indicated primary antibodies as follows: anti-MIF (MAB19781, R&D Systems Inc., Minneapolis, MN, USA), anti-eotaxin (ab25086) and anti-IL-4 (ab9728) (abcam Ltd, Cambridge, UK) and anti-MALT1 (sc-130494), EIF3F (sc-390413), IL-5 (sc-7887), IFN- γ (sc-9344-R) and β -actin (sc-8432) (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) antibodies. The relative amounts of proteins associated with specific antibodies were normalized according to the intensities of β -actin. Enhanced chemiluminescence (ECL) detection reagent (Luminata TM Forte Western HRP substrate, Millipore, Billerica, MA, USA) was used to detect chemiluminescent horseradish peroxidase (HRP)-conjugated antibodies. Chemiluminescent signals were detected using an LAS-4000 (Fuji Film, Tokyo, Japan). Band intensity was measured using the Image J software program and normalized for β -actin.

2.10. Measurement of histamine

The MC/9 murine mast cells were cultured with 500 ng/ml monoclonal anti-2,4-dinitrophenol-IgE in DMEM for 24 h. After washing twice with the culture medium, MC/9 cells were incubated with DNP-BSA (100 ng/ml) and berberine (100 μ M) either alone or in combination for 2 h. Histamine levels in release media were measured. The concentration of the released histamine was measured with the histamine enzyme immunoassay kit (Bertin Pharma, Montigny-Le Bretonneux, France) according to the manufacturer's recommendation.

2.11. GeneChip analyses

The gene expression was analyzed using a GeneChip® microarray system with a Mouse Genome 430 2.0 array (Affymetrix, Inc., Santa Clara, CA, USA) spotted with 45,101 probe sets. Samples for array hybridization were prepared as described in the Affymetrix GeneChip® Expression Technical Manual. The obtained hybridization intensity data were analyzed using GeneSpring® GX (Agilent Technologies, Inc.) to extract the significant genes. To examine the gene ontology, including the biological processes, cellular components, molecular functions and gene networks, the obtained data were analyzed using Ingenuity® Pathway Analysis tools (Ingenuity Systems Inc., Mountain View, CA, USA), as previously described. The microarray data "Identification of genes involved in the anti-inflammatory effects of berberine in DNP-activated mouse MC/9 mast cells" are accessible through GEO2R series accession number GSE76202.

2.12. Statistical analyses

The values are expressed as the means $\pm$ standard deviation (SD) of the respective test or control group. The statistical significance of differences between the control and test groups was evaluated by either Student's *t*-test or Dunnett's multiple comparisons. All statistical analyses were conducted under the blind condition.

3. Results

3.1. The effects of berberine on atopy-like symptoms in mice

In comparison to healthy mice grown under SPF condition, the

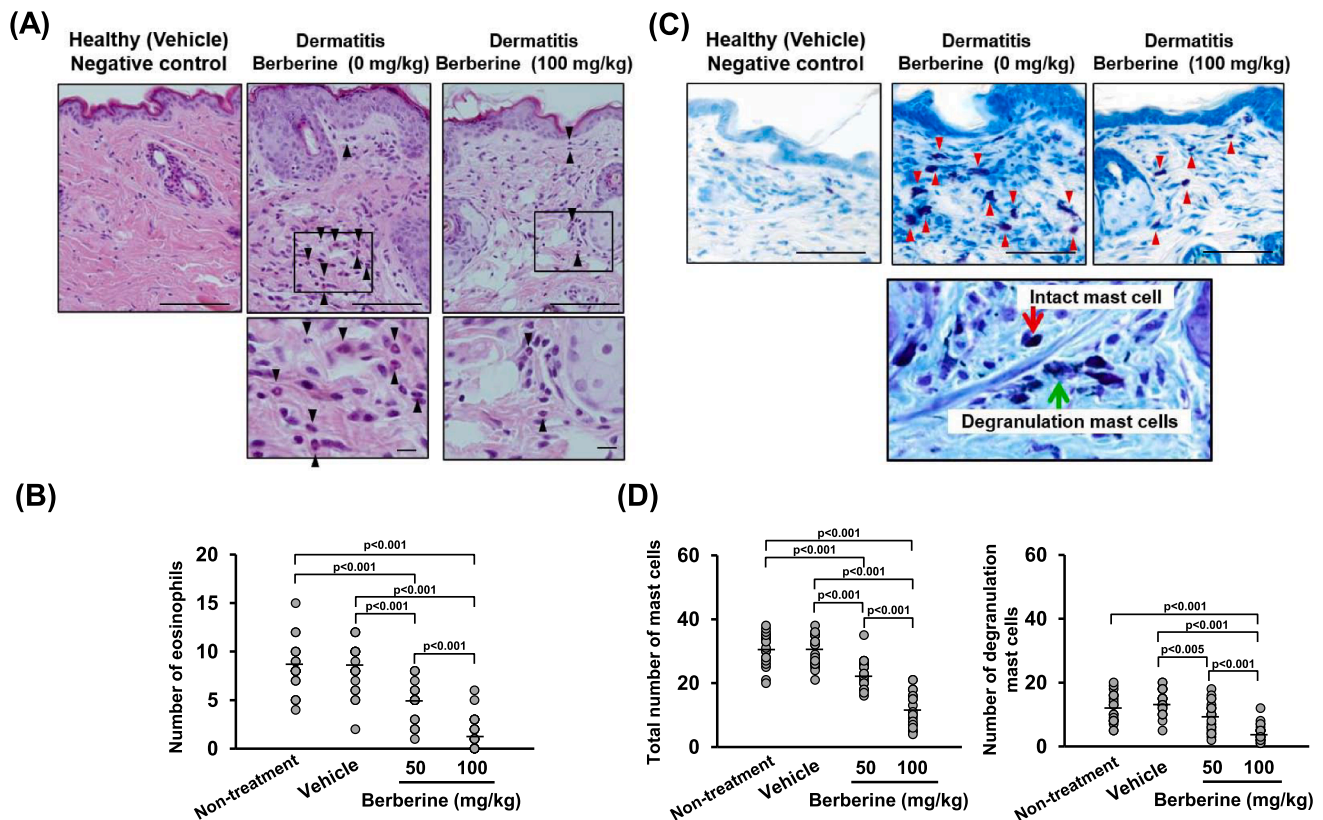


Fig. 2. The effects of berberine on the histological features in NC/Nga mice. The histological features of the NC/Nga mice to which berberine or vehicle (tap water) was orally administered were determined using H&E and toluidine blue staining. (A) Eosinophils are indicated by arrowheads. The experiments were repeated three times with similar results. One representative image is shown here. Scale bar for the large upper panels = 100 μm; scale bar for the small lower panels = 15 μm. (B) The number of eosinophils in the skin lesions of dermatitis NC/Nga mice to which vehicle or berberine was orally administered. Each value represents the mean ± SD (n = 5). P value shows statistical significance. (C) The histological assessment of the mast cells. The upper panel shows the presence of mast cells (red arrowheads). The lower panel, with a higher magnification view, shows non-degranulated (intact) mast cells (red arrow) and de-granulated mast cells (green arrow) in vehicle-treated dermatitis NC/Nga mice. Scale bar = 100 μm. (D) The total number of mast cells and the number of degranulated mast cells in the skin lesions of dermatitis NC/Nga mice to which vehicle or berberine was orally administered. Values are shown as the mean ± SD (n = 5). (B, D) P value shows statistical significance. The data shown are representative of three independent experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

clinical skin severity score (Fig. 1B), spontaneous scratching (an itch-related behaviour) (Fig. 1C) and serum IgE level (Fig. 1D) were apparently increased in dermatitis mice grown under conventional conditions with mites. The intermittent oral administration of berberine (50 and 100 or 100 mg/kg) in healthy mice did not affect the clinical skin severity score (Fig. 1B), spontaneous scratching (Fig. 1C), or serum IgE level (Fig. 1D). In comparison to vehicle-treated dermatitis mice, berberine (50 and 100 or 100 mg/kg) significantly inhibited the increased clinical skin severity score (Fig. 1B), spontaneous scratching (Fig. 1C), and the serum IgE level (Fig. 1D) in dermatitis mice.

3.2. The effects of berberine on the number of cutaneous eosinophils and mast cells in dermatitis mice

In comparison to healthy mice, the number of cutaneous eosinophils was apparently increased in dermatitis mice (Fig. 2A). The intermittent oral administration of berberine (50 and 100 mg/kg) significantly inhibited the increase of cutaneous eosinophils in dermatitis mice in a dose-dependent manner (Fig. 2A, B).

The number of cutaneous mast cells in dermatitis mice were apparently increased in comparison to healthy mice (Fig. 2C). The intermittent oral administration of berberine (50 and 100 mg/kg) significantly inhibited the increase of cutaneous mast cells in dermatitis mice in a dose-dependent manner (Fig. 2C, D). In addition, berberine (50 and 100

mg/kg) significantly attenuated the number of degranulated cutaneous mast cells in dermatitis mice in a dose-dependent manner (Fig. 2C, D).

3.3. The effects of berberine on the expression and production of eotaxin and pro-inflammatory cytokines in the skin of dermatitis mice

The mRNA (Fig. 3A) and protein (Fig. 3B,C) levels of eotaxin, Th2-type cytokines (MIF, IL-4 and IL-5) and Th1-type cytokine IFN-γ in the skin of dermatitis NC/Nga mice were apparently increased in comparison to healthy mice. The intermittent oral administration of berberine (50 and 100 mg/kg) did not affect the mRNA or protein levels of eotaxin, MIF, IL-4, IL-5 and IFN-γ in the skin of healthy mice. However, in the skin of dermatitis mice, berberine (50 and 100 mg/kg) inhibited the mRNA (Fig. 3A) and protein (Fig. 3B,C) levels of eotaxin, MIF, IL-4 and IL-5, but not IFN-γ.

3.4. The effect of berberine on the expression of eotaxin induced by IL-4 or/and MIF in primary cultures of murine fibroblasts

Eotaxin is regarded as a key mediator of tissue eosinophilia [21]. In primary cultures of murine fibroblasts, a single stimulation with IL-4 (1 ng/ml) or MIF (100 ng/ml) significantly increased the expression of *eotaxin* mRNA (Fig. 4A) and markedly increased the expression of the protein (Fig. 4B) in comparison to unstimulated fibroblasts.

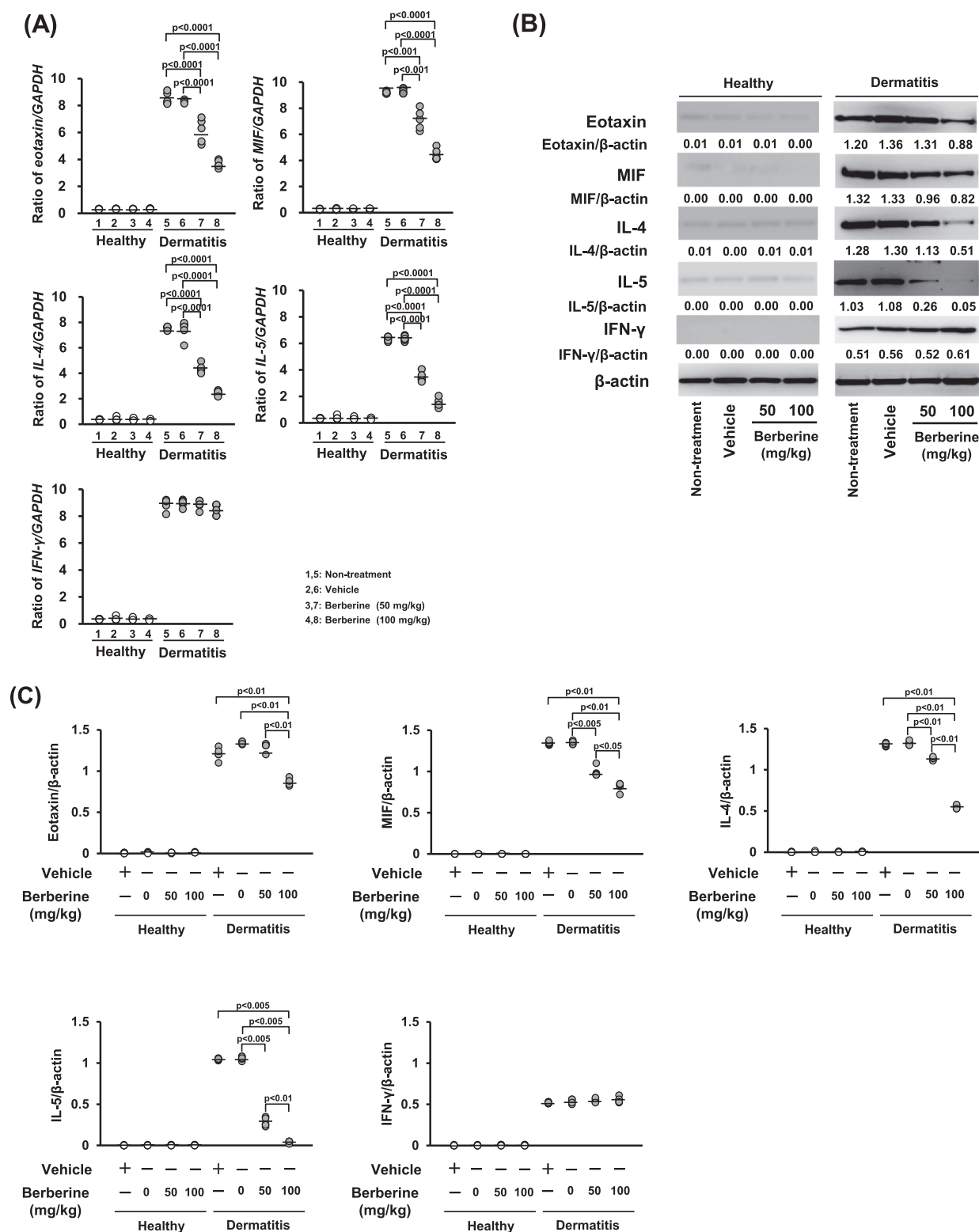


Fig. 3. The effects of berberine on the expression of eotaxin and pro-inflammatory cytokines in NC/Nga mouse skin. Berberine or vehicle (tap water) was orally administered 3 times a week for 12 days in healthy and dermatitis NC/Nga mice. Total RNA for real-time PCR (A) and protein for Western blotting (B) were extracted on day 12, after starting the oral administration of berberine or the vehicle. (A) For real-time PCR, the expression of each mRNA was normalised to the level of *GAPDH* mRNA in each sample. Data are presented as the mean $\pm$ SD ($n = 5$). P value shows statistical significance. The data shown are representative of three independent experiments. (B) For Western blotting, band densities were measured using the ImageJ software program to evaluate the intensity of bands. The signal intensity was normalised to that of β -actin. (C) The effects of berberine on the expression of eotaxin and pro-inflammatory cytokines in NC/Nga mouse skin. The data correspond to (B). Data are presented as the mean $\pm$ SD ($n = 5$). P value shows statistical significance. The data shown are representative of three independent experiments.

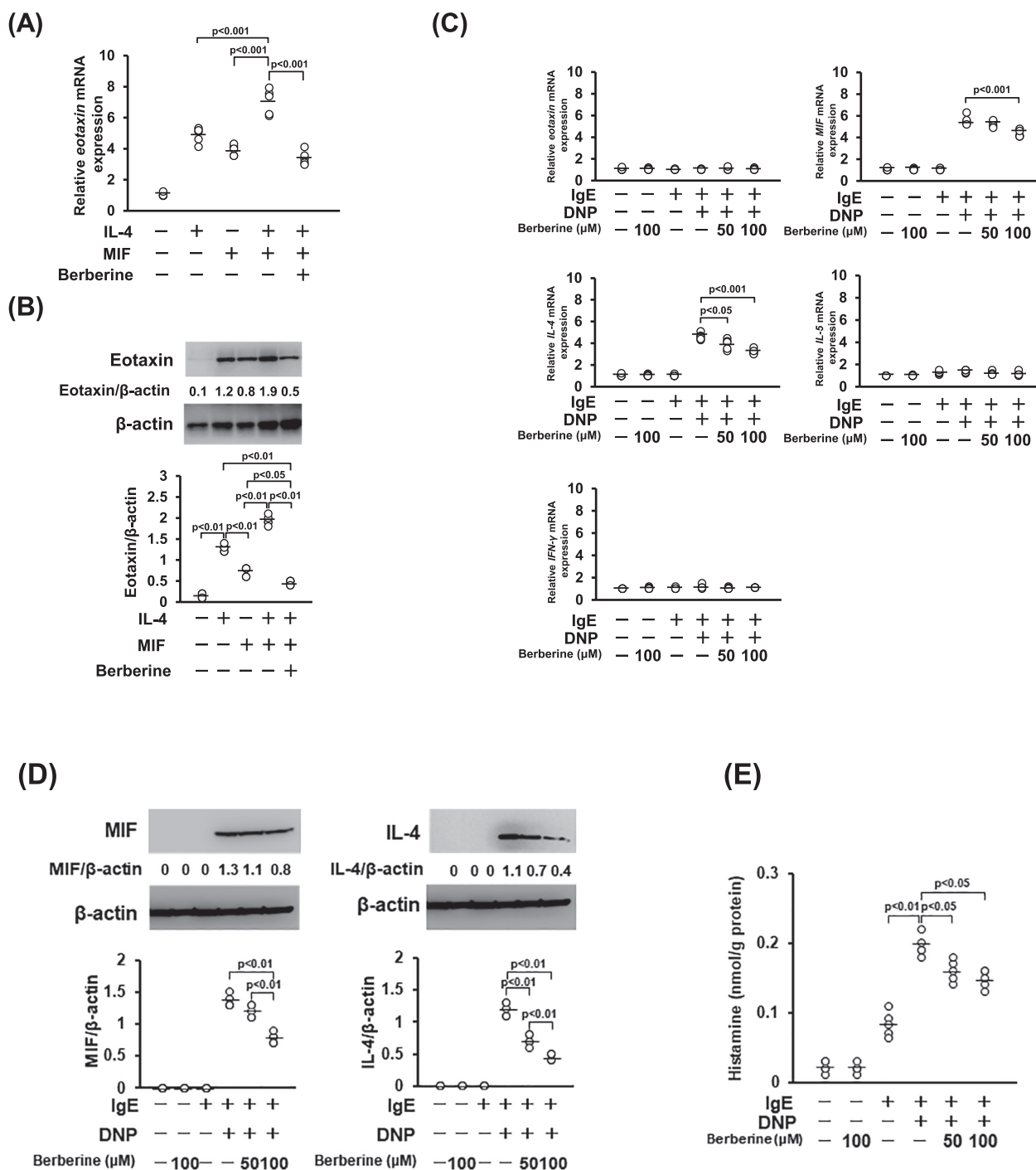


Fig. 4. The effects of berberine on the expression of eotaxin and pro-inflammatory cytokines (MIF, IL-4, IL-5 and INF- γ) in murine fibroblasts and mast cells, and on the release of histamine from mast cells. (A, B) Murine fibroblasts were stimulated with IL-4 (1 ng/ml), MIF (100 ng/ml) and berberine (10 μ M) alone or in combination. (C, D, E) Murine mast cells sensitized with anti-DNP-IgE were stimulated with DNP-BSA (100 ng/ml) and berberine (50 and 100 μ M). Total RNA (A, C) and protein (B, D) were extracted at 6 and 24 h after stimulation, respectively. The medium sample for measuring histamine was collected 120 min after stimulation. The mRNA (A, C) and protein (B, D) expression levels were analysed by real-time PCR and Western blotting method, respectively. For real-time PCR (n = 5), the relative amount of mRNA targeted was first normalised to the level of *GAPDH* mRNA in each sample and then to the average of expression levels in non-treated cells. For Western blotting (n = 5), the band densities were measured using the ImageJ software program to evaluate the intensity of bands. The signal intensity was normalised to that of β -actin. (B,D) Upper panels show typical examples on the results of Western blots. (E) Histamine in the medium (n = 5) was measured using an EIA kit. Data are presented as the mean $\pm$ SD. P value shows statistical significance. The data shown are representative of three independent experiments.

Furthermore, combined stimulation with MIF and IL-4 caused a substantial increase in the expression of eotaxin (mRNA and protein) (Fig. 4A, B). However, treatment with berberine (10 μ M) in the presence of MIF and IL-4 resulted in the decreased production of eotaxin (mRNA and protein) in fibroblasts in comparison to treatment with cytokines

either alone or in combination (Fig. 4A, B).

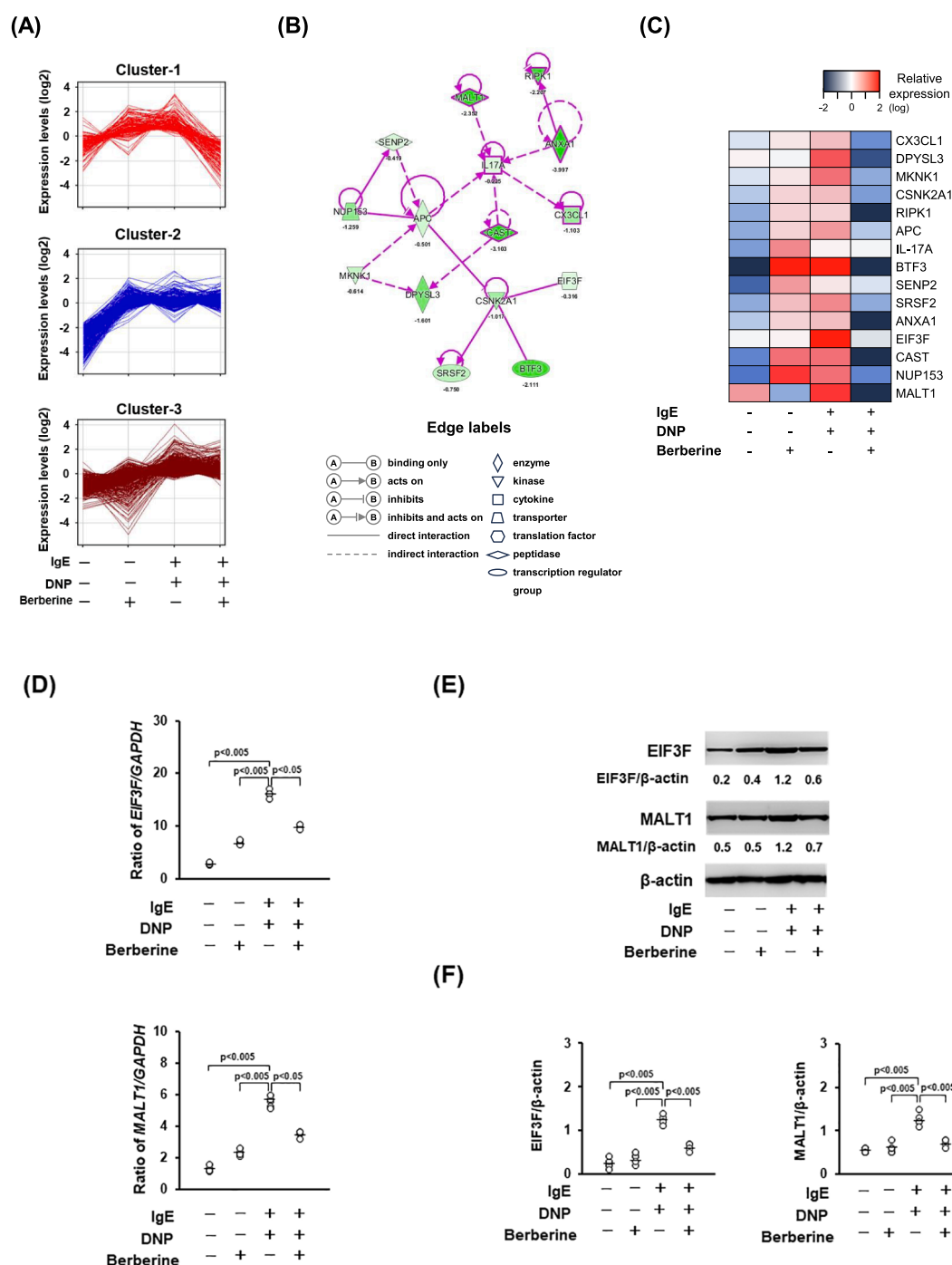


Fig. 5. A GeneChip analysis in mast cells exposed to DNP-induced allergic stimulation. (A) Mast cells sensitized with anti-DNP-IgE (500 ng/ml) were cultured with berberine (100 μ M) or DNP-BSA (100 ng/ml) or both for 24 h. K-means clustering of the probe sets that were differentially expressed by a factor of 2.0 or more was carried out using the GeneSpring[®] GX software program (Agilent Technologies Inc., Santa Clara, CA, USA). (B) Gene network cluster-1. Genes that were down-regulated in the berberine group in comparison to the DNP group in cluster-1 were analysed by the Ingenuity[®] Pathway analysis software program. The network is displayed graphically as nodes (genes) and edges (biological relationships between the nodes). Nodes and edges are displayed as various shapes and labels that depict the functional class of genes and the nature of the relationship between the nodes, respectively. (C) Heat maps of 15 selected genes. Intensity in the blue and red color range indicates downregulated and upregulated probe sets, respectively. Cells treated with berberine or DNP or both were used for the real-time PCR assay (D) and Western blotting assay (E, F) of the genes and proteins of MALT and EIF3F, respectively. (D) For real-time PCR, the expression of each mRNA was normalized to the level of *GAPDH* mRNA in each sample. Data are presented as the mean $\pm$ SD ($n = 5$). P value shows statistical significance. The data shown are representative of three independent experiments. (E) For Western blotting, band densities were measured using the ImageJ software program to evaluate the intensity of bands. The signal intensity was normalized to that of β -actin. (F) The effects of berberine on the expression of MALT and EIF3F in NC/Nga mouse skin. The data correspond to (E). Data are presented as the mean $\pm$ SD ($n = 5$). P value shows statistical significance. The data shown are representative of three independent experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

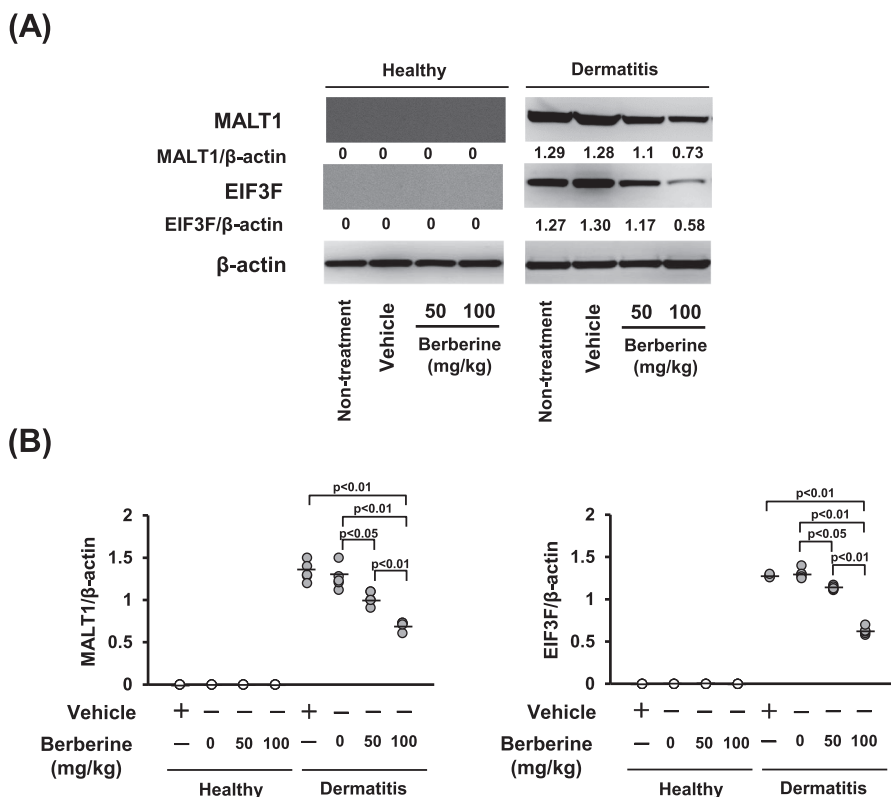


Fig. 6. The effects of berberine on the expression of MALT and EIF3F protein in NC/Nga mouse skin. Berberine or vehicle (tap water) was orally administered 3 times a week for 12 days in healthy and dermatitis NC/Nga mice. The protein for Western blotting were extracted on day 12, after starting the oral administration of berberine or the vehicle. Band densities were measured using the ImageJ software program to evaluate the intensity of bands. The signal intensity was normalized to that of β -actin. (A) show that the typical examples on the results of Western blots. (B) The data correspond to (A). Data are presented as the mean $\pm$ SD (n = 5). P value shows statistical significance. The data shown are representative of three independent experiments.

3.5. The effects of berberine on the DNP-induced MIF and IL-4 expression and histamine release in murine mast cells sensitized with anti-DNP IgE

After IgE-dependent stimulation, mast cells release allergic mediators, such as histamine, β -hexosaminidase, cytokines, chemokines and arachidonic acid derivatives, mediating chronic inflammation [22]. We therefore evaluated the effects of berberine on the expression and production of MIF and IL-4 in murine mast cells. Stimulation with berberine or sensitization with anti-DNP IgE did not induce the expression of eotaxin, MIF, IL-4, IL-5, or INF- γ at either the mRNA or protein level (Fig. 4C, D). In murine mast cells sensitized with anti-DNP IgE, DNP-BSA (100 ng/ml), the mRNA and protein expression of MIF and IL-4 were increased (Fig. 4C, D). However, DNP-BSA (100 ng/ml) did not affect the mRNA expression of eotaxin, IL-5, or INF- γ (Fig. 4C, D). Berberine (50 and 100 μ M) significantly inhibited the mRNA expression of MIF and IL-4 (Fig. 4C). Berberine also apparently inhibited the protein expression of MIF and IL-4 (Fig. 4D).

Berberine (100 μ M) itself did not release histamine from mast cells (Fig. 4E). In non-sensitized murine mast cells, IgE tended to increase the release of histamine, but not to a significant extent (Fig. 4E). In murine mast cells sensitized with anti-DNP IgE, DNP-BSA (100 ng/ml) significantly induced the release of histamine and the release was inhibited by berberine (50 and 100 μ M) (Fig. 4E).

3.6. GeneChip analysis of the DNP-induced mRNA expression in anti-DNP IgE-sensitized mast cells treated with or without berberine

A GeneChip analysis was performed to compare the gene expression between the DNP-BSA (100 ng/ml)-stimulated group and the group receiving combined treatment with DNP and berberine (100 μ M) using RNA isolated from cells from each group at 6 h after treatment. As shown in Fig. 5A, the differentially expressed probe sets were classified into 3 clusters, designated as Clusters-1, 2 and 3 by GeneSpring® GX software program. In Cluster-1, especially, berberine combination group 133 showed a rapid decrease in the probe sets in comparison to the DNP-

stimulated group. Furthermore, to determine the biologically relevant network and pathway of the genes related to inflammation, a pathway analysis of the down-regulated and up-regulated genes was carried out using the Ingenuity Pathways Analysis Knowledge Base (Fig. 5B, C). We selected automatically 15 genes (CX3CL1, DPYSL3, MKNK1, CSNK2A1, RIPK1, APC, IL-17A, BTF3, SENP2, SRSF2, ANXA1, AIF3F, CAST, NUP153, MALT1) related to inflammation using the software (Fig. 5B, C). Furthermore, we chose two genes (EIF3F and MALT1) that berberine alone did not affect the expression, were markedly increased in the expression by DNP-stimulation, and was suppressed by berberine (Fig. 5B, C).

To confirm the results of the gene chip analysis, a real-time qPCR assay was performed for two selected genes in the network (EIF3F and MALT1). Consistent with the gene chip analysis results, the expression of two selected genes was significantly increased by DNP stimulation compared to berberine (100 μ M) alone and without stimulation. However, in the presence of berberine, the expression of these genes was significantly decreased (Fig. 5C). Western blot assay also showed that berberine inhibited these protein (EIF3F and MALT1) expression (Fig. 5E and F). Furthermore, it was confirmed that the expression of EIF3F and MALT1 protein was apparently increased in the skin of dermatitis NC/Nga mice, compared with the skin of healthy NC/Nga mice (Fig. 6). The intermittent oral administration of berberine (50 and 100 mg/kg) inhibited the expression of these proteins in the skin of dermatitis NC/Nga mice significantly, compared with vehicle-treated dermatitis NC/Nga mice (Fig. 6).

3.7. EIF3F and MALT1 specific siRNA suppressed DNP-induced MIF and IL-4 expression in anti-DNP IgE-sensitized mast cells

To confirm the involvement of EIF3F and MALT1 in the DNP-induced MIF and IL-4 expression, we performed *in vitro* experiments using specific EIF3F and MALT1 siRNAs. Treatment with both specific siRNAs resulted in marked decreases in the EIF3F (Fig. 7A,B) and MALT1 (Fig. 7C,D) protein expression levels relative to the control and DNP-

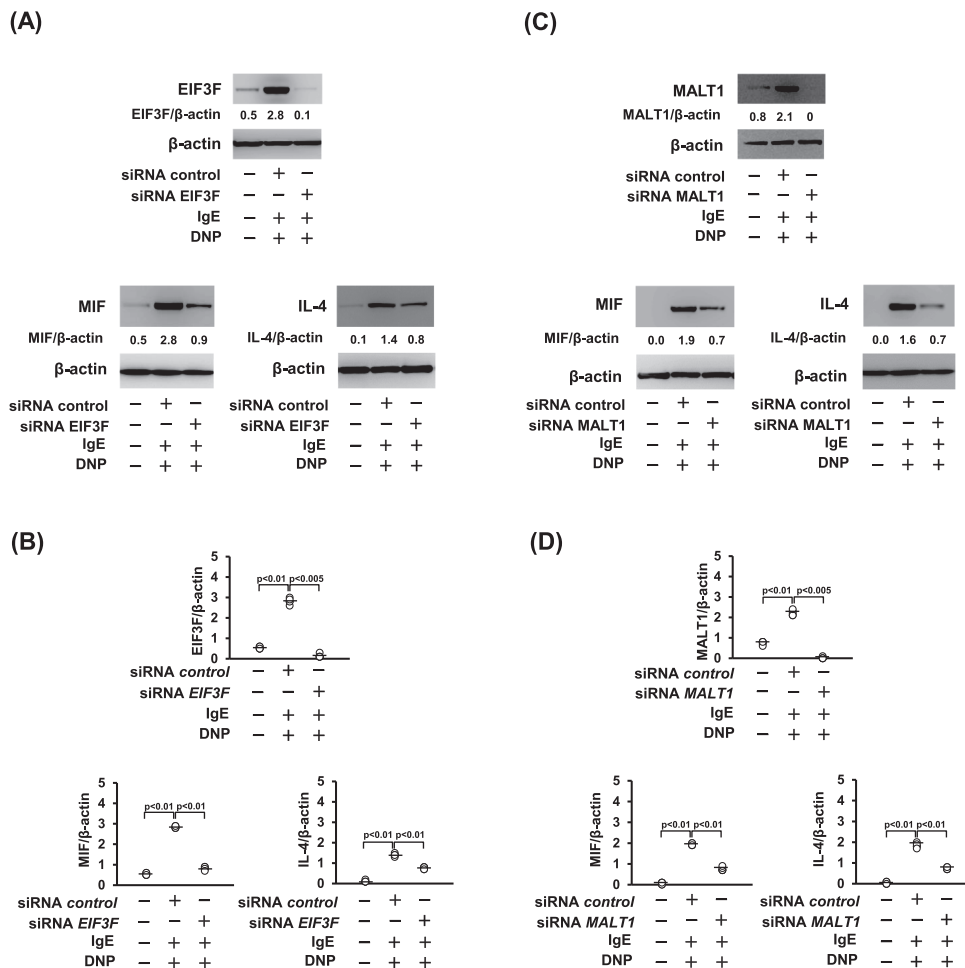


Fig. 7. The effects of *EIF3F* and *MALT1* siRNA on the expression of DNP-induced MIF and IL-4 in cultured murine mast cells. DNP-BSA (100 ng/ml) was applied to anti-DNP-IgE (500 ng/ml)-sensitized MC/9 cells treated with siRNA (30 pmol/ml) for *EIF3F* (A,B), *MALT1* (C,D), or nonspecific control (A ~ D). The protein levels of *EIF3F*, *MALT1*, MIF, IL-4, or β-actin were analysed by Western blotting. β-actin was detected as an internal control for Western blotting. Band densities were measured using the ImageJ software program to evaluate the intensity of bands. The signal intensity was normalised to that of β-actin. (A,C) show the typical examples on the results of Western blots. (B) and (D) correspond to (A) and (C), respectively. Data are presented as the mean ± SD (n = 5). *P* value shows statistical significance. The data shown are representative of three independent experiments.

stimulated groups. In addition, stimulation with DNP substantially induced the production of MIF and IL-4 proteins in comparison to unstimulated cells. However, in the presence of *EIF3F* (Fig. 7A,B) and *MALT1* (Fig. 7C,D) siRNAs, the DNP-induced expression of the MIF and IL-4 proteins was markedly suppressed.

4. Discussion

Itching is also an essential symptom of AD. The lesions are often erythematous with oedematous, weeping papules and vesicles in patients with acute AD, while thickened plaques of lichenification are observed in patients in the chronic stage of the disease. Mast cells produce several itch mediators (e.g., histamine [23] and tryptase [24,25]). Eosinophils not only produce itch mediators (e.g. platelet-activating factor [26]) but also activate mast cells, cross-talk between both cells takes place through an array of soluble mediators and ligands interactions that enhances their function [27]. In the skin lesions of AD patients [28,29] and dermatitis mice [16,30], the numbers of mast cells and eosinophils are increased. Therefore, the increase in these cells in AD skin may lead to the induction of itching and skin manifestations. Berberine is a well-known principal common active ingredient of *Coptidis rhizome* and *Phellodendri cortex* [3]. In the present study, the intermittent oral administration of berberine resulted in the significant improvement of itching and skin manifestation in dermatitis mice. In addition, berberine also inhibited the recruitment of inflammatory cells (eosinophils and mast cells). IL-4 released from Th2 cells [31] and mast cells [32] is known to increase the recruitment of mast cells [33]. In addition to mast cells [34], MIF is produced by other cells (e.g. fibroblasts and keratinocytes) in the skin [35]. The endogenous expression of

MIF is well known to play a role in mast cell recruitment [36]. IL-5 released from Th2 cells directly induces eosinophil infiltration [37,38]. In murine fibroblasts, IL-4/MIF increases the expression of eotaxin [39], which is a chemokine that increases eosinophil infiltration [40–42]. In the present study, we observed that berberine suppressed the expression of eotaxin and Th2-type cytokines (IL-4 and MIF) in murine fibroblasts and mast cells, respectively. However, no expression was observed for IL-5, and Th1-type cytokines INF-γ in mast cells. In AD-like lesion skin, berberine also suppressed the expression of eotaxin and Th2-type cytokines (IL-4, IL-5, and MIF), but not Th1-type cytokine INF-γ. These findings suggest that berberine regulates these changes through its action on Th2 cells, mast cells and fibroblasts and inhibits the recruitment of mast cells and eosinophils into the skin. Therefore, the inhibition of these cells by berberine is likely involved in the anti-pruritic and anti-inflammatory mechanisms. A recent study demonstrated that IL-4 is also an itch mediator [43]. Therefore, IL-4 elicits not only inflammatory cell recruitment but also itching.

IgE plays an important role in AD symptoms, especially the activation of mast cells. In AD patients and AD mice [16,17], the serum level of IgE is elevated. In the present study, berberine attenuated the serum IgE level in dermatitis mice. Berberine also suppressed the expression of IL-4 in the skin of dermatitis mice. IL-4 induces IgE production in B cells, and IL-5 enhances IL-4-induced IgE production [44,45]. Therefore, it is evident that the suppression of the IL-4 and IL-5 expression by berberine leads to a decrease in the serum IgE level in dermatitis mice and subsequently prevents the degranulation of mast cells.

In the present study, berberine reduced the expression of IL-4 and MIF in murine mast cells following DNP-induced allergic stimulation. In addition, berberine also attenuated the expression of eotaxin in murine

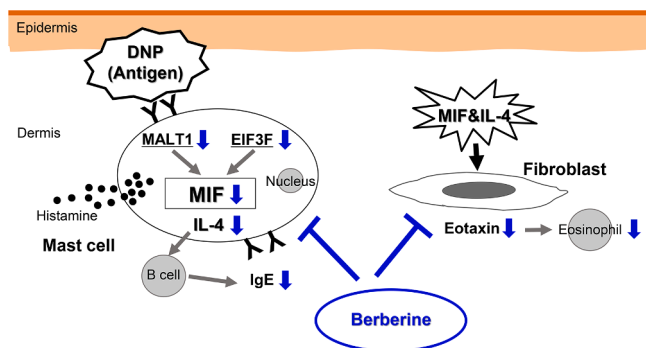


Fig. 8. A schematic illustration of the molecular mechanism underlying the DNP-stimulated induction of cytokines in mast cells by berberine. The down-regulation of EIF3F and MALT1 by berberine inhibits the expression of MIF and IL-4. The regulation of the IL-4 expression by berberine attenuates the production of IgE in B cells. Berberine also inhibits the MIF- and IL-4-induced eotaxin production in cutaneous fibroblasts. Taken together, the application of berberine leads to an anti-inflammatory effect by suppressing the expression of cytokines.

fibroblasts stimulated with IL-4/MIF. These findings suggest that IL-4 and MIF released from mast cells induces the production of eotaxin by fibroblasts that berberine inhibits both cells, leading to the inhibition of eotaxin production for the recruitment of eosinophils.

Furthermore, to confirm the regulation of the gene expression by berberine, we analysed the inflammation-related genes following a GeneChip analysis using the total RNA of murine mast cells. It was found that two genes (*EIF3F* and *MALT1*) were regulated highly by berberine. The increased expression of EIF3F and MALT1, which were regulated by berberine, was also observed in the skin of dermatitis NC/Nga mice. EIF3F is involved in several steps in the initiation of protein synthesis [46–48]. Deubiquitination by EIF3F is necessary for the nuclear import of activated Notch [49]. Notch signalling enhances the FcεRI-mediated cytokine (e.g. IL-4) production by mast cells [50]. MALT1 controls antigen receptor-mediated signalling to nuclear factor-κB (NF-κB) [51]. Although NF-κB is activated during IgE-mediated allergic inflammation, the activation of NF-κB is not necessary for the production of IL-4 [52]. However, NF-κB plays an important role in the expression of MIF [53]. The involvement of these highly regulated genes was further confirmed using specific siRNAs. Interestingly, treatment with specific siRNAs for *EIF3F* and *MALT1* markedly suppressed the DNP-induced production of MIF and IL-4 in mast cells. Taken together, these findings suggest that the DNP-induced upregulation of the *MALT1* and *EIF3F* genes, which are related to inflammation, is responsible for the increased production of MIF and IL-4. In this study, berberine inhibited the expression of MALT1 and EIF3F and subsequently suppressed the production of MIF and IL-4, suggesting that the regulation of MALT1 and EIF3F is involved in the atopic dermatitis effects of berberine. However, the detail mechanisms on the regulation of MALT1 and EIF3F by berberine still remains unclear. Therefore, we should dissolve this issue in a future study.

We conclude that the regulation of the EIF3F and MALT1 expression by berberine is involved in the inhibitory effects on eotaxin, IL-4 and MIF and that these mechanisms are also involved in the anti-atopic dermatitis effects of berberine (Fig. 8).

CRediT authorship contribution statement

Authors' Contributions Conceptualization: All authors. Funding acquisition: T.A., Y.Y., T.S. Investigation, Methodology: T.A., Y.Y., Y.T., T.S. Project administration, Resources, Software, and Supervision: T.A., Y.Y., T.S. Data curation and Formal analysis: All authors. Writing-original draft, Writing-review & editing: All authors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- [1] J.M. Hanifin, G. Rajika, Diagnostic features of atopic dermatitis, *Acta. Dermat. Venerol. (Stockh)* 92 (1980) 44–47.
- [2] D.Y. Leung, M. Boguniewicz, M.D. Howell, I. Nomura, Q.A. Hamid, New insights into atopic dermatitis, *J. Clin. Invest.* 113 (2004) 651–657.
- [3] N. Ikarashi, M. Tajima, K. Suzuki, T. Toda, K. Ito, W. Ochiai, K. Sugiyama, Inhibition of preadipocyte differentiation and lipid accumulation by Oregandokuto treatment of 3T3-L1 cultures, *Phytother. Res.* 26 (2012) 91–100.
- [4] M.J. Ko, J.H. Baek, A clinical study on the effect of Hwangryunhaedock-tang on atopic dermatitis, *J. Korean Orient. Pediatr.* 26 (2012) 51–60.
- [5] T. Shimizu, Efficacy of kampo medicine in treating atopic dermatitis: an overview, *Evid-based Comp. Alt. Med.* 2013 (2013) 1–5.
- [6] F. Cheng, Y. Wang, J. Li, C. Su, F. Wu, W.H. Xia, Z. Yang, B.B. Yu, Y.X. Qiu, J. Tao, Berberine improves endothelial function by reducing endothelial microparticles-mediated oxidative stress in humans, *Int. J. Cardiol.* 167 (2013) 936–942.
- [7] H.W. Jeong, K.C. Hsu, J.W. Lee, M. Ham, J.Y. Huh, H.J. Shin, W.S. Kim, J.B. Kim, Berberine suppresses proinflammatory responses through AMPK activation in macrophages, *Am. J. Physiol. Endocrinol. Metab.* 296 (2009) 955–964.
- [8] C.L. Kuo, C.W. Chi, T.Y. Liu, The anti-inflammatory potential of berberine in vitro and in vivo, *Cancer Lett.* 203 (2004) 127–137.
- [9] B.J. Zhang, D. Xu, Y. Guo, J. Ping, L.B. Chen, H. Wang, Protection by and anti-oxidant mechanism of berberine against rat liver fibrosis induced by multiple hepatotoxic factors, *Clin. Exp. Pharmacol. Physiol.* 35 (2008) 303–309.
- [10] P. Jabbarzadeh Kaboli, A. Rahmat, P. Ismail, K.H. Ling, Targets and mechanisms of berberine, a natural drug with potential to treat cancer with special focus on breast cancer, *Eur. J. Pharmacol.* 740 (2014) 584–595.
- [11] K. Yasukawa, M. Takido, T. Ikekawa, F. Shimada, M. Takeuchi, S. Nakagawa, Relative inhibitory activity of berberine-type alkaloids against 12-O-tetradecanoylphorbol-13-acetate-induced inflammation in mice, *Chem. Pharm. Bull.* 39 (1991) 1462–1465.
- [12] K. Fukuda, Y. Hibiya, M. Mutoh, M. Koshiji, S. Akao, H. Fujiwara, Inhibition by berberine of cyclooxygenase-2 transcriptional activity in human colon cancer cells, *J. Ethnopharmacol.* 66 (1999) 227–233.
- [13] W. Li, F. Liu, J. Wang, M. Long, Z. Wang, MicroRNA-21-mediated inhibition of mast cell degranulation involved in the protective effect of berberine on 2,4-dinitrofluorobenzene-induced allergic contact dermatitis in rats via p38 pathway, *Inflammation* 41 (2018) 689–699.
- [14] S. Kim, Y. Kim, J.E. Kim, K.H. Cho, J.H. Chung, Berberine inhibits TPA-induced MMP-9 and IL-6 expression in normal human keratinocytes, *Phytomedicine* 15 (2008) 340–347.
- [15] M.S. Tsang, D. Jiao, B.C. Chan, K.L. Hon, P.C. Leung, C.B. Lau, E.C. Wong, L. Cheng, C.K. Chan, C.W. Lam, C.K. Wong, Anti-inflammatory activities of pentaherbs formula, berberine, gallic acid and chlorogenic acid in atopic dermatitis-like skin inflammation, *Molecules* 21 (2016) 519.
- [16] H. Matsuda, N. Watanabe, G.P. Geba, J. Sperl, M. Tsudzuki, J. Hiroi, M. Matsumoto, H. Ushio, S. Saito, P.W. Askenase, C. Ra, Development of atopic dermatitis-like skin lesion with IgE hyperproduction in NC/Nga mice, *Int. Immunol.* 9 (1997) 461–466.
- [17] T. Yamaguchi, T. Maekawa, Y. Nishikawa, H. Nojima, M. Kaneko, T. Kawakita, T. Miyamoto, Y. Kuraishi, Characterization of itch-associated responses of NC mice with mite-induced chronic dermatitis, *J. Dermatol. Sci.* 25 (2001) 20–28.
- [18] Y. Yoshihisa, T. Andoh, K. Matsunaga, M.U. Rehman, T. Maoka, T. Shimizu, P. Bobé, Efficacy of astaxanthin for the treatment of atopic dermatitis in a murine model, *PLoS One* 11 (3) (2016) e0152288, <https://doi.org/10.1371/journal.pone.0152288>.
- [19] T. Andoh, S. Haza, A. Saito, Y. Kuraishi, Involvement of leukotriene B₄ in spontaneous itch-related behaviour in NC mice with atopic dermatitis-like skin lesions, *Exp. Dermatol.* 20 (2011) 894–898.
- [20] T. Shimizu, R. Abe, A. Ohkawara, Y. Mizue, J. Nishihira, Macrophage migration inhibitory factor is an essential immunoregulatory cytokine in atopic dermatitis, *Biochem. Biophys. Res. Commun.* 240 (1997) 173–178.
- [21] N. Terada, N. Hamano, T. Nomura, T. Numata, K. Hirai, T. Nakajima, H. Yamada, O. Yoshie, T. Ikeda-Ito, A. Konno, Interleukin-13 and tumour necrosis factor-α synergistically induce eotaxin production in human nasal fibroblasts, *Clin. Exp. Allergy* 30 (2000) 348–355.
- [22] S.J. Galli, M. Tsai, IgE and mast cells in allergic disease, *Nature Med.* 18 (2012) 693–704.

- [23] H. Eppinger, Über eine eigentümliche Hautreaktion, hervorgerufen durch Ergamin, *Wie. Med. Wschr.* 63 (1913) 141.
- [24] M. Steinhoff, U. Neisius, A. Ikoma, M. Fartasch, G. Heyer, P.S. Skov, T.A. Luger, M. Schmelz, Proteinase-activated receptor-2 mediates itch: a novel pathway for pruritus in human skin, *J. Neurosci.* 23 (2003) 6176–6180.
- [25] H. Ui, T. Andoh, J.B. Lee, H. Nojima, Y. Kuraishi, Potent pruritogenic action of tryptase mediated by PAR-2 receptor and its involvement in anti-pruritic effect of nafamostat mesilate in mice, *Eur. J. Pharmacol.* 530 (2006) 172–178.
- [26] B. Fjellner, O. Hägermark, Experimental pruritus evoked by platelet activating factor (PAF-acether) in human skin, *Acta Derm. Venereol.* 65 (1985) 409–412.
- [27] N. Landolina, R.S. Gangwar, F. Levi-Schaffer, Mast cells' integrated actions with eosinophils and fibroblasts in allergic inflammation: implications for therapy, *Adv. Immunol.* 125 (2015) 41–85.
- [28] K.D. Cooper, Atopic dermatitis: recent trends in pathogenesis and therapy, *J. Invest. Dermatol.* 102 (1) (1994) 128–137.
- [29] S. Pastore, F. Mascia, M.L. Giustizieri, A. Giannetti, G. Girolomoni, Pathogenetic mechanisms of atopic dermatitis, *Arch. Immunol. Ther. Exp.* 48 (2000) 497–504.
- [30] K. Tsujii, T. Andoh, H. Ui, J.B. Lee, Y. Kuraishi, Involvement of tryptase and proteinase-activated receptor-2 in spontaneous itch-associated response in mice with atopy-like dermatitis, *J. Pharmacol. Sci.* 109 (2009) 388–395.
- [31] G. Del Prete, Human Th1 and Th2 lymphocytes: their role in the pathophysiology of atopy, *Allergy* 47 (1992) 450–455.
- [32] M.A. Brown, J.H. Pierce, C.J. Watson, J. Falco, J.N. Ihle, W.E. Paul, B cell stimulatory factor-1/interleukin-4 mRNA is expressed by normal and transformed mast cells, *Cell* 50 (1987) 809–818.
- [33] N. Olsson, D.D. Taub, G. Nilsson, Regulation of mast cell migration by TH1 and TH2 cytokines: identification of tumour necrosis factor-alpha and interleukin-4 as mast cell chemotaxins, *Scan. J. Immunol.* 59 (2004) 267–272.
- [34] H. Chen, M. Centola, S.F. Altschul, H. Metzger, Characterization of gene expression in resting and activated mast cells, *J. Exp. Med.* 188 (1998) 1657–1668.
- [35] T. Shimizu, Role of macrophage migration inhibitory factor (MIF) in the skin, *J. Dermatol. Sci.* 37 (2) (2005) 65–73.
- [36] J. Pölajeva, T. Bergström, P.H. Edqvist, A. Lundequist, A. Sjösten, G. Nilsson, A. Smits, M. Bergqvist, F. Pontén, B. Westermarck, G. Pejler, K. Forsberg Nilsson, E. Tchougounova, Glioma-derived macrophage migration inhibitory factor (MIF) promotes mast cell recruitment in a STAT5-dependent manner, *Mol. Oncol.* 8 (2014) 50–58.
- [37] P.D. Collins, S. Marleau, D.A. Griffiths, P.J. Jose, T.J. Williams, Cooperation between interleukin-5 and the chemokine eotaxin to induce eosinophil accumulation in vivo, *J. Exp. Med.* 182 (1995) 1169–1174.
- [38] C.J. Sanderson, Interleukin-5, eosinophils, and disease, *Blood* 79 (1992) 3101–3109.
- [39] Y. Yoshihisa, T. Makino, K. Matsunaga, A. Honda, O. Norisugi, R. Abe, H. Shimizu, T. Shimizu, Macrophage migration inhibitory factor is essential for eosinophil recruitment in allergen-induced skin inflammation, *J. Invest. Dermatol.* 131 (2011) 925–931.
- [40] P.S. Foster, A.W. Mould, M. Yang, J. Mackenzie, J. Mattes, S.P. Hogan, S. Mahalingam, A.N. Mckenzie, M.E. Rothenberg, I.G. Young, K.I. Matthaai, D. Webb, Elemental signals regulating eosinophil accumulation in the lung, *Immunol. Rev.* 179 (2001) 173–181.
- [41] J.C. Gutierrez-Ramos, C. Lloyd, J.A. Gonzalo, Eotaxin: from an eosinophilic chemokine to a major regulator of allergic reactions, *Immunol. Today* 20 (1999) 500–504.
- [42] C.M. Lloyd, T. Delaney, T. Nguyen, J. Tian, A.C. Martinez, A.J. Coyle, J. C. Gutierrez-Ramos, CC chemokine receptor (CCR)3/eotaxin is followed by CCR4/monocyte-derived chemokine in mediating pulmonary T helper lymphocyte type 2 recruitment after serial antigen challenge in vivo, *J. Exp. Med.* 191 (2000) 265–274.
- [43] M. Campion, L. Smith, S. Gatault, C. Métais, J. Buddenkotte, M. Steinhoff, Interleukin-4 and interleukin-13 evoke scratching behaviour in mice, *Exp. Dermatol.* 28 (2019) 1501–1504.
- [44] J. Pène, F. Rousset, F. Brière, I. Chrétien, J.Y. Bonnefoy, H. Spits, T. Yokota, N. Arai, K. Arai, J. Banchereau, IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons gamma and alpha and prostaglandin E₂, *Proc. Natl. Acad. Sci. USA* 85 (1988) 6880–6884.
- [45] J. Pène, F. Rousset, F. Brière, I. Chrétien, J. Wideman, J.Y. Bonnefoy, J.E. De Vries, Interleukin 5 enhances interleukin 4-induced IgE production by normal human B cells. The role of soluble CD23 antigen, *Eur. J. Immunol.* 18 (1988) 929–935.
- [46] A.S. Lee, P.J. Kranzusch, J.H. Cate, eIF3 targets cell-proliferation messenger RNAs for translational activation or repression, *Nature* 522 (2015) 111–114.
- [47] A.S. Lee, P.J. Kranzusch, J.A. Doudna, J.H. Cate, eIF3d is an mRNA cap-binding protein that is required for specialized translation initiation, *Nature* 536 (2016) 96–99.
- [48] M. Masutani, N. Sonenberg, S. Yokoyama, H. Imataka, Reconstitution reveals the functional core of mammalian eIF3, *EMBO J.* 26 (2007) 3373–3383.
- [49] Julien Moretti, Patricia Chastagner, Stefano Gastaldello, Sara F. Heuss, Annette M. Dirac, René Bernards, Maria G. Masucci, Alain Israël, Christel Brou, Norbert Perrimon, The translation initiation factor 3f (eIF3f) exhibits a deubiquitinase activity regulating Notch activation, *PLoS Biol.* 8 (11) (2010) e1000545, <https://doi.org/10.1371/journal.pbio.1000545>.
- [50] N. Nakano, C. Nishiyama, H. Yagita, M. Hara, Y. Motomura, M. Kubo, K. Okumura, H. Ogawa, Notch signaling enhances FcεRI-mediated cytokine production by mast cells through direct and indirect mechanisms, *J. Immunol.* 194 (2015) 4535–4544.
- [51] Mathijs Baens, Luca Bonsignore, Riet Somers, Charlotte Vanderheydt, Stephen D. Weeks, Jenny Gunnarsson, Ewa Nilsson, Robert G. Roth, Margot Thome, Peter Marynen, Marek Cebecauer, MALT1 auto-proteolysis is essential for NF-κB-dependent gene transcription in activated lymphocytes, *PLoS One* 9 (8) (2014) e103774, <https://doi.org/10.1371/journal.pone.0103774>.
- [52] D.L. Marquardt, L.L. Walker, Dependence of mast cell IgE-mediated cytokine production on nuclear factor-kappaB activity, *J. Allergy Clin. Immunol.* 105 (2000) 500–505.
- [53] W.G. Cao, M. Morin, V. Sengers, C. Metz, T. Roger, R. Maheux, A. Akoum, Tumour necrosis factor-alpha up-regulates macrophage migration inhibitory factor expression in endometrial stromal cells via the nuclear transcription factor NF-kappaB, *Human Reprod.* 21 (2006) 421–428.