The Inhibitory Effect of Premature *Citrus unshiu* Extract on Atopic Dermatitis *In Vitro* and *In Vivo*

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Atopic dermatitis (AD) is a chronic, recurrent inflammatory skin disease that is associated with Th2-cell-mediated allergy. The process that leads to infiltration of inflammatory cells into an AD lesion is remarkably dependent on various chemokines, especially TARC (thymus and activation-regulated chemokine/CCL17) and MDC (macrophage-derived chemokine/CCL22). Serum levels of these chemokines are over-expressed in AD patients. *Citrus unshiu*, which is known as Satsuma mandarin, has anti-oxidative, anti-inflammation, and anti-microbial activity. Therefore, we investigated the effect of EtOH extract of premature *C. unshiu* on AD. We did this using a DNCB-induced AD mouse model. We also tried to confirm an inhibitory effect for premature *C. unshiu* on the expression of inflammatory chemokines in IFN-γ and TNF-α stimulated HaCaT human keratinocytes. We found that extract of premature *C. unshiu* reduced DNCB-induced symptoms such as hyperkeratosis, increased skin thickness, and infiltrated mast cells, in our AD-like animal model. The extract decreased levels of IFN-γ and IL-4 in ConA-stimulated splenocytes isolated from DNCB-treated mice. Also, extract of premature *C. unshiu* inhibited mRNA expression and protein production of TARC and MDC through the inhibition of STAT1 phosphorylation. These results suggest that *C. unshiu* has anti-atopic activity by regulating inflammatory chemokines such as TARC and MDC.

**Key words**: DNCB, Atopic dermatitis, Hairless mouse, TARC/CCL17, MDC/CCL22, *Citrus unshiu*

**INTRODUCTION**

Atopic dermatitis (AD) is a chronic inflammatory skin disease that develops on an allergic and genetic background. It is characterized by pruritic and eczematous lesions, increased serum IgE levels, and the infiltration into lesional skin of inflammatory cells such as Th2-type cells, eosinophils, mast cells, and macrophages (Abramovits, 2005; Bonness and Bieber, 2007). AD is assumed to be an aspect of the Th2 condition due to increases in Th2 cytokines such as IL-4 and IL-5 in acute phase skin lesions, and to be a mixed condition of Th1/Th2 in the chronic phase skin lesions (e.g., IFN-γ, IL-12, and GM-CSF). Moreover, it has been shown that changes in expression of various inflammatory chemokines produced by several cell types, including CCL1, CCL2, CCL5, CCL11, CCL13, CCL18, CCL20, CCL26, and CCL27, are similar to the AD phenotype (Imai *et al*., 1999).

Chemokines are a group of a small cytokines produced by various cell types and are divided into C, CC, CXC, and CX3C subfamilies based on NH₂-terminal cysteine-motifs. The main role of chemokines is regulation of dendritic cell (DC) trafficking and recruitment of inflammatory cells such as T cells, eosinophils, macrophages to sites of infection and inflammation (Pease and Williams, 2006). In particular, thymus-regulated and activation-regulated chemokines (TARC and CCL17) and macrophage-derived chemokines (MDC and CCL22) are produced by keratinocytes, dendritic cells, endothelial cells, and bronchial epithelial cells, and play important roles in the recruitment of Th2-type cells expressing CC chemokine receptor 4 (CCR4) on their surfaces. Previous studies showed that serum levels of these Th2-type chemokines are increased in patients with AD (Hijnen *et al*., 2004; Imai *et al*., 1999; Kakinuma *et al*., 2002; Leung *et al*., 2003).

Recently, many types of mouse models similar to our AD model have been developed. In one, the ears or the dorsal skin of a BALB/c mouse is repeatedly exposed to dinitrochlorobenzene (DNCB) or trinitrochlorobenzene (TNCB) (Kitagaki *et al*., 1995; Lee *et al*., 2010). Another is NCGa mice that is developed AD-like symptoms under conventional condition, but not specific pathogen-free (SPF) con-
ditions (Jin et al., 2009; Vestergaard et al., 1999). However, each mouse model has disadvantages. For example, in the first model, the ear surface area may be too small; or one has to shave or clip their fur prior to various assays which interferes with time-course studies due to hair re-growth. In contrast, the model of AD in which a hairless mouse is given repeated topical applications of TNCB is more similar to the features of human skin and is useful for doing non-invasive biophysical and histological studies (Matsumoto et al., 2004, 2005; Qi et al., 2009).

Citrus unshiu is a kind of citrus fruit in the Rutaceae family. C. unshiu is composed of rind and sarcocarp and includes various bioactive substances such as essential oils, carotenoids, cellulose, pectin, limonoid, etc. In recent studies, it has been reported that flavonoids are also contained in C. unshiu extracts which have anti-oxidant, anti-cancer, and anti-inflammatory activity. Also, the flavonoid content changes during maturation of C. unshiu and is high in premature C. unshiu (Kang et al., 2005; Kim et al., 2009).

Here, we aimed to find the effect of premature C. unshiu on AD-like markers. We investigated the effects of premature C. unshiu extract on TARC and MDC, important markers of AD. Furthermore, we examined the effect of premature C. unshiu extract in a DNBC-challenged animal model.

MATERIALS AND METHODS

Cell culture and reagents. An immortalized human keratinocyte cell line, HaCaT, was cultured in DMEM supplemented with 10% FBS and 100 U/ml penicillin-streptomycin in a humidified CO2 incubator. HaCaT keratinocytes were provided by Prof. Moon Je Cho (Department of Biochemistry, Jeju National University, Korea).

Recombinant human interferon-γ (hIFN-γ) and human tumor necrosis factor-α (hTNF-α) were obtained from GIBCO (Grand Island, NY). Fetal bovine serum (FBS), Dulbecco’s modified Eagle’s medium (DMEM), and RPMI1640 were obtained from GIBCO. Dinitrochlorobenzene (DNCB) was purchased for the Tokyo chemical industry (Japan). Concanavalin A (ConA) was purchased from Sigma (St. Louis, MO). Enzyme-linked immunosorbent assay (ELISA) kits for human TARC and MDC or mouse IL-4 and IFN-γ were obtained from R&D Systems (St. Louis, MO). TARC/CCL17 and MDC/CCL22 primers were obtained from Bioneer (Korea). β-actin primers were purchased from Bionex (Korea). Antibodies against phospho-STAT1, STAT1, and β-actin were purchased from Cell Signaling (Beverly, MA), Becton Dickinson (San Diego, CA), and Sigma respectively. All other reagents were of reagent grade.

Preparation of C. unshiu extract. The dried powder (37.95 kg) of C. unshiu was extracted with 80% ethanol (475 l) for 4 hr at 60°C and the extract was incrasated by rotary evaporator for 3 hr. To remove the ethanol, water (20 l) and extract were mixed and incrassated again. Subsequently, extracted liquid was filtered through filter paper and frozen on a freezing tray for 48 hr. Freeze drying for 60 hr afforded a perfectly dried extract (7.95 kg) of C. unshiu which was dissolved in EtOH for experiments.

Experimental animals and DNBC application. Female, 7-week-old SKH1-hairless mice (25 ± 2 g) were purchased from Orient Bio (Korea) and were maintained for 1 week before the start of any experiments. These mice were housed in a conventional animal room under controlled temperature (23 ± 1°C), humidity (60 ± 10%) and light (light on from 08:00 to 20:00 hr). They were fed a standard laboratory diet. Water was given ad libitum. Mice were divided into four groups (normal, induction, positive control, and CU groups; n = 5/group). The whole dorsal skins of 15 mice were sensitized to DNBC by application of 100 µl of 1% DNBC in acetone (day −7). Seven days later, 100 µl of 0.5% DNBC was applied to the dorsal skin (day 0). The application was repeated every 2 days for up to 36 days. EtOH extract of premature C. unshiu (CU) was applied at 2-day intervals from day 16. For a positive control group, HYDROCORT cream (Green Cross, Korea) containing 2 mg/g hydrocortisone valerate was used. This animal study was approved by the Animal Care and Use Committee at Jeju National University.

Measurement of Skin-fold thickness and histological analysis. Skin-fold thickness was measured with a Digital Thickness Gauge (Mitutoyo, Japan) by pulling up the skin from shoulder to hip. Mice were sacrificed at the end of the experiment. The skin biopsies were fixed with 10% buffered formalin and then embedded in paraffin. Paraffin sections (3 µm each) were stained with hematoxylin and eosin solution for detecting skin histological feature and various inflammatory cells and toluidine blue solution for detecting mast cells.

Spleen cell culture. Mice from each group were sacrificed by cervical dislocation and their spleens were removed aseptically. To produce single-cell suspensions, spleens were forced through wire gauzes (70 µm pore size) using the plunger from a 5 ml syringe. Red blood cells (RBC) were removed using RBC lysis buffer. After washing, the splenocytes were seeded (5.0 × 10⁶ cells/ml) using trypan blue assays and incubated in the presence of concanavalin A (2 µg/ml) without any drugs for 6 days. Following incubation, the supernatants were collected to determine levels of IL-4 and IFN-γ.

Cell viability assay. Cell viability was determined using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assays. Briefly, HaCaT cells were stimulated with IFN-γ and TNF-α in the absence or presence of extract

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of premature *C. unshiu*. After incubating for 24 hr, cells were treated with 20 µl MTT for 4 hr. The formazan precipitate was dissolved in 200 µl of dimethyl sulphoxide (DMSO) for 30 min, and the absorbance of the contents of each well was measured at 540 nm using a microplate reader.

**ELISA.** Production of IL-4, IFN-γ, TARC and MDC proteins in the supernatant were measured with ELISA kits according to the manufacturer’s instructions.

**Extraction of total RNA and RT-PCR.** Total RNA was isolated using easy-Blue™ Total RNA Extraction kit (iNtRON Biotechnology, Korea) according to the manufacturer’s instructions. Reverse transcription was performed using a First-Strand cDNA Synthesis kit (Promega). Briefly, total RNA (1 µg) was incubated with oligo(dT)₁₅ primer at 70°C for 5 min and cooled on ice for 5 min. After addition of the RT premix, reactions were incubated at 42°C for 60 min. Reactions were terminated by raising the temperature to 70°C for 15 min. The PCR reaction was conducted using i-Taq™ DNA polymerase (iNtRON Biotechnology) with the appropriate sense and antisense primers for TARC, MDC, and β-actin. The primers used in this experiment are shown in Table 1. PCR was performed using a C1000 (Bio-Rad, Hercules, CA) and thermal cycling conditions were set to 94°C for 30 sec, annealing at 55~60°C for 30 sec, and extending at 72°C for 2 min, repeated 30~35 times, and followed by incubation at 72°C for 10 min. The reaction products were visualized by electrophoresis on a 1.2% agarose gel (Promega) and UV light illumination after staining with ethidium bromide. The relative intensity was analyzed using Quantity One software version 4.2.1.

**SDS-PAGE and western blot analysis.** HaCaT cells were washed twice with ice-cold PBS and then disrupted in lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 % Nonident P-40, 2 mM EDTA, 1 mM EGTA, 1 mM NaVO₃, 10 mM NaF, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 25 µg/ml leupeptin] on ice for 30 min. The cell lysates were centrifuged at 15,000 rpm for 15 min at 4°C and supernatants were used for western blotting. Total protein concentration of each sample was quantified by the Bio-Rad assay method (Bio-Rad, Hercules, CA). Extracts containing 30 µg of protein were separated on an 8% sodium dodecylsulfate (SDS) polyacrylamide gel and transferred onto a polyvinylidene fluoride (PVDF) membrane. The membrane was blocked with blocking buffer (5% skim milk in TTBS) for 2 hr at room temperature, followed by incubation with rabbit anti-p-STAT1 (1 : 1000), mouse anti-STAT1 (1 : 1000), and mouse anti-β-actin (1 : 2500) antibodies overnight at 4°C. All antibodies were diluted in 1% BSA in TTBS buffer. After washing, the membrane was incubated with horseradish peroxidase (HRP)-conjugated anti-mouse IgG and anti-rabbit IgG diluted 1 : 5000 for 1 hr at RT. The result was visualized with a western blot detection system (iNtRON Biotechnology, Korea) according to the manufacturer’s instructions.

**Statistical analysis.** Quantity One version 4.2.1 and Image-Pro plus version 4.5 software were used to transform images into numerical values. Student’s t-test and two-way analysis of variance were used to determine the statistical significance of differences between values for the experimental and control groups. Data represent the mean ± standard error.

**RESULTS**

**Extract of premature *C. unshiu* reduced symptoms in DNCB-challenged hairless mice.** We identified the effect of premature *C. unshiu* using DNCB-challenged AD-like mice. To the naked eye, 3% *C. unshiu* application resulted, on day 36, in a reduction of symptoms such as wrinkles and redness in the dorsal skin of DNCB-treated mice (Fig. 1A~D). Histopathological analysis was done using H&E staining and toluidine blue staining on day 36. In the Normal group, no histopathological change was observed. In the DNCB-treated group, there was marked hyperkeratosis and epidermal hyperplasia. DNCB-induced histological abnormalities were reduced in DNCB-treated mice given 3% *C. unshiu* (Fig. 1E~H, I~L). Also, the skin-fold thickness of the CU group was significantly reduced compared to the Induction group (Fig. 2).

**Extract of premature *C. unshiu* reduced the production of Th1 and Th2 cytokines by splenocytes from DNCB-challenged mice.** Next, we analyzed the effect of premature *C. unshiu* on the production of cytokines induced

| Gene  | Primer sequences | Fragment siz (bp) |
|-------|------------------|------------------|
| TARC  | Sense 5'-ATGGCCCCCACTGAAGATGCT-3' | 351 |
|       | Antisense 5'-TGAACACCAACGGTGGAGGT-3' | |
| MDC   | Sense 5'-GCATGGCTCGCTACAGACT-3', 5'-GCAGGAGGGAGGAGAGGA-3' | 497 |
| β-actin | Sense 5'-ATGGGTGTCAGAAGGATT-CCTATG-3' | 588 |
by modulation of the Th1 and Th2 cytokine milieu in splenocytes isolated from DNCB-challenged mice. In the presence of ConA, levels of IL-4 (22.4 ± 1.4 pg/ml) and IFN-γ (227.6 ± 15.8 pg/ml) was increased in splenocytes from the Induction group. However, EtOH extract of premature C. unshiu reduced the production of IL-4 (2.64 ± 0.9 pg/ml) and IFN-γ (no detection). HYDCORT cream, which was used as a positive control, also reduced levels of both cytokines, although the decrease in IL-4 by HYDCORT cream was not statistically significant (Fig. 3).

**Extract of premature C. unshiu inhibited expression of mRNA and protein for TARC and MDC in IFN-γ and TNF-α-stimulated HaCaT cells.** To identify the effect of premature C. unshiu on cell viability in IFN-γ and TNF-α-stimulated HaCaT human keratinocytes, cell viability was examined using MTT assay. As shown in Fig. 4, premature C. unshiu extract had no effect on the viability of HaCaT cells. EtOH extract of premature C. unshiu did inhibit mRNA levels of TARC and MDC (Fig. 5 & 6). Treatment with

![Fig. 1. Direct visual observations of skin surface features (A–D), histological features of skin tissue stained with hematoxylin and eosin (×200) (E–H) or toluidine blue (×200) (I–L) in DNCB-challenged hairless mice (Day 36) treated with Citrus unshiu (CU). A, E, I: Normal; B, F, J: Induction; C, G, K: Positive cont.; D, H, L: 3% CU.](image)

![Fig. 2. Effect of premature Citrus unshiu on skin-fold thickness in DNCB-challenged hairless mice. Each bar graph represents mean ± SD for five mice. * P < 0.05.](image)
Effect of Premature *Citrus unshiu* on Atopic Dermatitis Model

EtOH extract of premature *C. unshiu* dose-dependently inhibited the induction of TARC and MDC protein (Fig. 7) in supernatants from cultured HaCaT cells that had been stimulated with IFN-γ and IFN-γ for 24 hr. Mouse IL-4 and IFN-γ product from splenocytes were determined using ELISA. **, P < 0.005.

**Fig. 3.** Effect of premature *Citrus unshiu* on mouse IL-4 or IFN-γ production by ConA-stimulated splenocytes from each experimental group. Mouse splenocytes were isolated from spleens of each group. Cells (5.0 × 10⁵ cells/ml) were then pre-incubated for 24 hr and stimulated with ConA (2 µg/ml) for 6 days. Mouse IL-4 and IFN-γ production from splenocytes were determined using ELISA. **, P < 0.005.

**Fig. 4.** Effect of premature *Citrus unshiu* on cell viability in IFN-γ and TNF-α-stimulated HaCaT human keratinocytes. HaCaT cells (3.0 × 10⁵ cells/ml) were pre-incubated for 18 hr. Cells were then stimulated with IFN-γ (10 ng/ml) and TNF-α (10 ng/ml) in the presence of premature CU (25, 50, 100, or 200 µg/ml) for 24 hr. Cell viability was analyzed by MTT assay. The error bars indicate standard deviation. Each measurement was performed in triplicate.

**Fig. 5.** Effect of premature *Citrus unshiu* on mRNA expression of TARC in HaCaT human keratinocytes. HaCaT cells (5.0 × 10⁵ cells/ml) were pre-incubated for 18 hr. mRNA expression of the TARC was determined from the 18 hr culture of cells stimulated by IFN-γ (10 ng/ml) and TNF-α (10 ng/ml) in the presence of premature CU in a dose-dependent manner (25, 50, 100, or 200 µg/ml).

**Fig. 6.** Effect of premature *Citrus unshiu* on mRNA expression of MDC in HaCaT human keratinocytes. HaCaT cells (5.0 × 10⁵ cells/ml) were pre-incubated for 18 hr. mRNA expression of the MDC was determined from the 18 hr culture of cells stimulated by IFN-γ (10 ng/ml) and TNF-α (10 ng/ml) in the presence of premature CU in a dose-dependent manner (25, 50, 100, or 200 µg/ml).

IFN-γ stimulates the activation of its receptor and the phosphorylation of Jak5 that are combined with receptor. Subsequently, STAT1 phosphorylated by Jaks is translocated from cytosol to nucleus and acts as a transcription factor. TARC and MDC production in IFN-γ and/or TNF-α-stimulated HaCaT cells was increased by activation of Jak-STAT signaling (Ju et al., 2009; Qi et al., 2009, 2011). Therefore, we examined whether EtOH extract of premature *C. unshiu* inhibited phosphorylation of STAT1 in HaCaT cells. We found that IFN-γ and TNF-α-stimulation induced the phosphorylation of STAT1 in HaCaT cells after 30 min; EtOH

**Extract of premature *C. unshiu* reduced TARC and MDC expression through inhibition of STAT1 phosphorylation in IFN-γ- and TNF-α-stimulated HaCaT cells.** Various cytokines act through signaling pathway of their receptors,
Effect of premature C. unshiu on IFN-γ and TNF-α induced TARC and MDC protein productions in HaCaT human keratinocytes. HaCaT cells (3.0 × 10⁵ cells/ml) were pre-incubated for 18 hr and TARC and MDC expression were determined from the culture supernatant of cells stimulated by IFN-γ (10 ng/ml) and TNF-α (10 ng/ml) with the indicated concentrations of premature CU (25, 50, 100, or 200 µg/ml) for 24 hr. Protein levels of TARC and MDC were measured by ELISA. The error bars indicate standard deviation. #, P < 0.05; ##, P < 0.01; *, P < 0.005.

Effect of premature C. unshiu on phosphorylation of STAT1 by IFN-γ and TNF-α in HaCaT human keratinocytes. HaCaT cells (5 × 10⁵ cells/ml) were pre-incubated for 18 hr. The cells were pre-treated with premature CU over a range of doses (25, 50, 100 µg/ml) for 2 hr and stimulated by IFN-γ (10 ng/ml) and TNF-α (10 ng/ml) for 30 min. Levels of phosphorylated or total proteins (pSTAT1 & STAT1) were determined using Western blotting.

DISCUSSION

Extract of premature C. unshiu not only inhibited the production of TARC and MDC in IFN-γ and TNF-α-stimulated HaCaT keratinocytes, but also, it reduced symptoms in our animal model of AD. C. unshiu is one of the most popular fruits in the world. The dried peel has been used in medicinal preparations in oriental medicine. There are many studies that show that components of C. unshiu are changed according to harvest date and that the premature fruit contains a higher percentage of flavonoids than the mature fruit (Kang et al., 2005; Kim et al., 2009). Premature C. unshiu contains flavonoids such as naringin, hesperidin, neohesperidin, narirutin, nobiletin, etc., that are known to have anti-inflammation, anti-oxidant, anti-diabetes, and anti-allergy effects in vitro and in vivo (Choi et al., 2007; Harasstani et al., 2010; Itoh et al., 2009; Lee et al., 2010; Murakami et al., 2000; Sakata et al., 2003).

Of various animal models, TNCB and DNCB-stimulated mice show diverse symptoms that are similar to those of AD, such as skin infiltration of inflammatory cells, and increases in mast cell numbers and in serum IgE levels (Kitagaki et al., 1995; Matsumoto et al., 2004; Qi et al., 2009). Therefore, we used the DNCB-challenged hairless mouse model to identify the effects of C. unshiu in vivo. To the naked eye, symptoms like hyperkeratosis, redness, and increases in skin thickness were reduced in DNCB-treated mice by treatment with premature C. unshiu. Furthermore, histological observation indicated that C. unshiu decreased the hyperkeratosis and the infiltration of inflammatory cells into the dermis of lesional skin. In general, depending on the disease phase of AD, the cytokine milieu is known to change during the acute phase, including increases in Th2 cell cytokines such as IL-4, IL-5 and IL-13; and during the chronic phase to Th1/0 cell cytokines such as IFN-γ, IL-12 and GM-CSF (Bonness and Bieber, 2007). Hence, we identified the effect of premature C. unshiu extract on production of IL-4 and IFN-γ in splenocytes stimulated with ConA, a T cell specific antigen. As Fig. 3 shows, productions of both cytokines were inhibited by extract of premature C. unshiu. This shows that premature C. unshiu may reduce AD symptoms by direct control of T cell activation.

AD is an inflammatory skin disease and is distinguished by infiltration of numerous inflammatory cells in to the site of inflammation (Abramovits, 2005; Bonness and Bieber, 2007). Several chemokines have been reported to participate in leukocyte recruitment to the lesional skin. Two of them, TARC and MDC, which are chemokines specific for Th2 cells, were increased in the serum of AD patients (Hijnen et al., 2004; Kakinuma et al., 2002; Leung et al., 2003; Nakazato et al., 2008). Therefore, we investigated the inhibitory effects of C. unshiu extract on levels of TARC and MDC in the IFN-γ and TNF-α-stimulated HaCaT keratinocytes. Premature C. unshiu suppressed mRNA expression and protein production of TARC and MDC (Fig. 5–7). Premature C. unshiu may therefore regulate the recruitment of Th2-type cells into lesions of AD by suppressing production of inflammatory chemokines related to AD.

IFN-γ acts through IFNGR1 and R2 and then activates JAK/STAT, ERK, p38 MAPK, and NF-xB pathways (Gough
et al., 2008). Ju et al. found that TARC production from IFN-γ and TNF-α stimulated HaCaT keratinocytes was decreased by treatment with Jak/STAT and NF-κB inhibitors. Qi et al. found that TARC and MDC production in IFN-γ stimulated HaCaT keratinocytes were inhibited by Jak inhibitor I, SB203580, and Bay11-7082, inhibitors of Jak1/2, p38 or NF-κB, respectively. The Janus family of tyrosine kinases (Jaks) is an integral factor in IFN-γ-activated signaling cascades and regulates tyrosine phosphorylation of STAT proteins. STAT protein translocation to the nucleus and binding to IFN-γ activated sequences (GAS) is a crucial component of IFN-γ signaling (Gough et al., 2008; Saha et al., 2010). Therefore, we examined the effects of premature C. unshiu on the phosphorylation of STAT1 by stimulation of IFN-γ and TNF-α. Extract of premature C. unshiu inhibited STAT1 phosphorylation after IFN-γ and TNF-α treatment at 30 min in a dose-dependent manner. However, further investigation is needed to determine whether the inhibitory effect of premature C. unshiu on STAT1 phosphorylation occurs through inhibition of Jak1/2 or through effects on other pathway components such as ERK, p38, and NF-κB. Also, we need to explore the effect of premature C. unshiu on suppressor of cytokine signaling proteins 1 (SOCS1) that is known to be a negative regulator and is an important component of the IFN-γ signaling pathway (Yoshimura et al., 2007).

In summary, premature C. unshiu moderates symptoms in DNCB-treated mice and suppresses the expression of TARC and MDC in HaCaT keratinocytes by suppressing STAT1 activation. The biological effects of premature C. unshiu confirmed in this study indicate that an extract of premature C. unshiu or a preparation of its components might be useful as an anti-atopic agent.

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