

Anti-inflammatory effects of aloe aptamers from *Aloe vera* and *Aloe ferox* varieties on human skin keratinocytes and their TDS and NDS applications

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Abstract

Atopic dermatitis, a chronic inflammatory skin disease, is characterized by severe itching of the skin and long-lasting eczematous lesions, which are thought to be a response to local allergens including house dust mites (HDM). Through previous studies, we confirmed that ALOVEtamer, a modified form of ALtamer (DNA fragment) composed of Aloe aptamers from *Aloe vera* and *Aloe ferox* varieties, specifically binds to derivative of viniferin extracts (VED) and increases stability and antioxidant effects. In addition, ALtamer has been shown to have an anti-inflammatory effect on skin inflammation through HDM mouse experiments, and it is being developed as an oral Nutrient Delivery System (NDS) and a skin Transdermal Delivery System (TDS) in the future. Oxidative stress is one of the major causes of inflammatory diseases including HDM-induced skin inflammation, suggesting that the antioxidant activity of ALOVEtamer can modulate the inflammatory response to HDM in the skin keratinocyte cell line HaCaT and primary skin keratinocytes. ALOVEtamer not only inhibited HDM-induced proliferation of both types of cells, but also suppressed HDM-induced increases in interleukin (IL)-1 α and IL-6 production by these cells. In addition, ALOVEtamer suppressed IL-17 and IL-22 production by T cells, which are closely related to the pathogenesis of atopic dermatitis, and HDM-induced IL-22R α expression. ALOVEtamer also reduced thymic and activation-regulating chemokine (TARC) production by inhibiting the interaction between IL-22 and IL-22R α and reducing T cell migration. HDM treatment significantly increased the expression of glial cell line-derived neurotrophic factor (GDNF), which is associated with pruritus in skin lesions of atopic dermatitis, but this increase was reduced by ALOVEtamer treatment. Taken together, these results suggest that ALOVEtamer, based on aloe ferox aptamer and viniferin extract, can effectively control inflammatory lesions such as atopic dermatitis by regulating the production of inflammatory cytokines and GDNF induced by HDM.

Keywords: atopic dermatitis; house dust mites; *Aloe ferox*; Aptamer; glial cell line-derived neurotrophic factor; oral Nutrient Delivery System (NDS), skin Transdermal Delivery System (TDS)

I. Introduction

Atopic dermatitis is a representative chronic inflammatory skin disease associated with itching, dry skin, and eczema. Atopic dermatitis is also often accompanied by respiratory diseases such as allergic rhinitis and asthma. Although the cause of atopic dermatitis has not yet been identified, several factors, including environmental, genetic, immunological abnormalities, and skin barrier disruption, are thought to be involved in the pathogenesis. In particular, keratinocyte dysfunction caused by house dust mites (HDM) is associated with skin inflammation in atopic dermatitis. This is because HDM treatment is associated with a decrease in antimicrobial peptides [1-3] as well as an increase in the production of inflammatory chemokines and thymus and activation-regulating chemokines (TARC) [4,5].

TARC production is induced by the interaction between interleukin (IL)-22 and its receptor IL-22R α , which in turn drives the migration of T cells to AD skin lesions. IL-22 is a proinflammatory cytokine produced primarily by CD4⁺ T cells and natural killer (NK) cells, but also has anti-inflammatory functions [6-8]. IL-22 levels are higher in the skin of patients with atopic dermatitis than in controls, and its production is increased by HDM [9-11]. Treatment with IL-22 and HDM extracts induces the production of IL-6, a proinflammatory

cytokine produced by peripheral blood T cells in patients with atopic dermatitis [4,12,13]. HDM extracts also increase the expression of IL-22R α , a heterodimer composed of IL-22R α and IL-10R β , which is highly expressed in the skin [4]. IL-22R α plays a pathogenic role in psoriatic skin by inducing differentiation and proliferation of keratinocytes [14–16]. Taken together, these results suggest that IL-22 and its receptor are potential therapeutic targets for regulating HDM-induced skin inflammation. Therefore, novel therapeutics that modulate the production and expression of IL-22 and its receptor may be very useful for treating HDM-induced skin inflammation in atopic dermatitis.

Nerve growth factor (NGF) released from keratinocytes is closely related to pruritus, the most difficult symptom associated with skin lesions of atopic dermatitis [17,18]. NGF plays an important role in the survival and differentiation of neurons and in the sensitization of nerve fibers [19–23]. Glial cell-derived neurotrophic factor (GDNF), a protein produced by glial cells, prevents the degeneration of dopaminergic neurons and increases the number and diameter of nerve fibers with high affinity for dopamine. Increased GDNF production in skin lesions of atopic dermatitis is a strong cause of pruritus [24]. This suggests that pruritus in skin lesions of inflammatory atopic dermatitis may be regulated by regulating GDNF production. However, no substance that effectively regulates GDNF production has been identified so far.

Since oxidative stress is a major cause of inflammatory diseases and skin aging, antioxidant molecules can effectively protect the skin from damage caused by reactive oxygen species (ROS) [25–27]. Previous studies have shown that *aloe ferox* aptamer (ALTamer) and a derivative of viniferin extract (VED) have protective effects against ROS-induced skin inflammation after UVB irradiation [28]. From these results, we confirmed that the efficacy of ALOVEtamer could be further increased by prolonging its antioxidant activity. ALTamer is a single-stranded DNA or RNA oligonucleotide that selectively binds to various molecules and has been identified as an aptamer that further stabilizes the VED [23,29,30].

It has been previously reported that ALTamer has an anti-inflammatory effect on skin inflammation through our previous study [25,31]. Oxidative stress is one of the major causes of inflammatory diseases including HDM-induced skin inflammation [25,27,32], which suggests that the antioxidant activity of ALOVEtamer may regulate the inflammatory response to HDM in human skin keratinocytes, HaCaT, and primary skin keratinocytes. Therefore, in this study, we evaluated the effect of ALOVEtamer on HDM-induced skin inflammation and the changes in inflammation-related molecules in HaCaT human keratinocyte cell line and primary keratinocytes.

In this study, we found that ALOVEtamer stimulated GDNF production in skin lesions of atopic dermatitis, thereby regulating IL-22 production and receptor expression, suggesting that ALOVEtamer, which has anti-itch and anti-inflammatory effects, has potential as a therapeutic agent for the treatment of atopic dermatitis patients when utilized as an oral Nutrient Delivery System (NDS) and a skin Transdermal Delivery System (TDS).

II. Materials and Methods

2.1. Cell Culture and Reagents

HaCaT human keratinocyte cell line provided by Seoul National University College of Pharmacy was cultured in RPMI1640 medium (Seoul, Korea) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Hyclone, Queensland, Australia) and antibiotics (100 U/mL penicillin and 100 μ g/mL streptomycin; WELGENE) at 37°C in a humidified 5% CO₂ incubator.

HDM extract of house dust mite *Dermatophagoides pteronyssinus* was purchased from Cosmo Bio Co. Ltd. (Tokyo, Japan). The mites were homogenized in 10-fold 50 mM phosphate buffer (pH = 7.2), and the homogenate was stirred at 4°C for 24 h. The centrifuged supernatant was dialyzed four times against PBS (Visking tube, 4°C).

2.2. Isolation and preparation of primary keratinocytes

Primary keratinocytes were isolated using foreskin provided by a healthy young female donor. The skin samples were cultured overnight at 4°C in a dispase solution (Dispase II; Thermo Fisher Scientific, Waltham, MA, USA). The epidermis was separated from the dermis, and epidermal cells were separated from the epidermis by treatment with 0.05% trypsin-EDTA (Invitrogen, Carlsbad, CA, USA) at 37°C for 15 min. After adding 10 mg/mL soybean trypsin inhibitor (Thermo Fisher Scientific), cells were pelleted, washed, and resuspended in keratinocyte serum-free medium (KBM; Thermo Fisher Scientific) containing human keratinocyte growth supplement (Thermo Fisher Scientific) and antibiotics (100 U/mL penicillin and 100 μ g/mL streptomycin; WELGENE) at 37°C in a humidified 5% CO₂ incubator. Cells used in this experiment were derived from passages 4–6 of cells grown in a monolayer and cultured until confluent. Fibroblast contamination of the keratinocyte cultures was determined by flow cytometry using a monoclonal anti-human fibroblast antibody (clone ASO₂). All volunteers provided written informed consent. The study protocol was approved by the Institutional Review Board (IRB) of Seoul National University Hospital (IRB number 24107-088-1008), and all experiments adhered to the principles of the Declaration of Helsinki.

2.3. Reduced graphene oxide (rGO)-based SELEX for ALtamer

Specific single-stranded DNA (ssDNA) ALtamer that selectively bind to VED were generated using the Systematic Evolution of Ligands by EXponential Enrichment (SELEX) method. The ssDNA aloe aptamer library, consisting of 30 randomly generated nucleotide sequences of 60 nucleotides each, was surrounded by primer sites (5-ATGCGGATCCCGCGC-(N)30-GCGCGAAGCTTGCGC-3) for amplification [33]. The ssDNA aptamer library underwent five rounds of enrichment, and the fourth round included a counter-selection process in an attempt to obtain more specific sequences. ssDNA library-bound rGO was placed in 1×binding reaction buffer (NaCl 135 mM, KCl 2.7 mM, Na₂HPO₄ 4.3 mM, KH₂PO₄ 1.4 mM, MgCl₂ 1 mM) and incubated for 30 min to immobilize the ssDNA library on the rGO. The mixture was centrifuged at 13,000 rpm for 15 min, and the supernatant was evaporated to remove the remaining unbound ssDNA. The ssDNA library-bound rGO pellet was first rinsed with 200 µL of 1×binding buffer and then centrifuged again under the same environment to remove all remaining unbound ssDNA. Then, 40 nmol of viniferine extract derivatives mixed in 200 µL of 1×binding buffer was added to the pellet. The viniferine extract derivatives and rGO mixture was incubated for 1 h to elute the target-binding aptamer from rGO. The eluted candidates were cloned through the same process incorporated in the construction of the ssDNA library. The course of this series was considered as a single round. The binding time and buffer concentration were adjusted in each round to select the sequence suitable for the target.

2.4. ALOVEtamer preparation

ALtamer was donated by Kim Jung-moon Aloe KJM Bio Research Institute (Seoul, Korea). It was dissolved in PBS containing 1 mM MgCl₂ and heated at 95°C for 5 min. It was then cooled to room temperature to allow the aptamer to fold into the tertiary structure. VED (Seoul, Korea) purchased from Korea University Guro Hospital was added to the DNA aptamer at a ratio of 1:50 (w/w).

2.5. Confocal microscopy

HaCaT and primary keratinocytes were cultured on 1 cm² coverslips for 24 h at 37°C in a 5% CO₂ atmosphere. After washing with PBS, HaCaT was treated with HDM extract (25 µg/mL), TNF-α, and IFN-γ (10 ng/mL) and cultured for 24 h. HaCaT grown on coverslips were fixed in 4% paraformaldehyde (PFA) and preincubated overnight with 5% goat serum in PBS-T (0.3% Triton X-100 in PBS). Cells were incubated with rabbit anti-human IL-22Rα antibody (Abcam, Cambridge, UK) and Alexa Fluor 633-conjugated anti-rabbit antibody (Invitrogen). Fluorescent images were captured with an inverted confocal microscope (FV3000; Olympus, Tokyo, Japan) after mounting with mounting solution containing DAPI (Immunobioscience, Mukilteo, WA, USA).

2.6. T cell isolation

Peripheral blood mononuclear cells (PBMCs) were obtained from healthy adult donors according to a protocol approved by the Seoul National University Hospital IRB (#2408-138-5812). T cells were purified from PBMCs using the MACS negative selection system and the Pan T cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany). The purity of T cells was determined by staining cells with APC-conjugated anti-CD3 antibody (e-Bioscience, San Diego, CA, USA), followed by flow cytometry and data analysis using FlowJo software (Tree Star, Ashland, OR, USA).

2.7. Enzyme-linked immunosorbent assay (ELISA)

Human T cells, HaCaT, and primary keratinocytes were treated with HDM extract (25 µg/mL) for 24, 48, or 72 h, respectively, and the concentrations of TNF-α, IFN-γ, IL-22, and GDNF in the cell supernatants were assessed by ELISA according to the manufacturer's instructions (R&D Systems). The optical density of each well was determined using SoftmaxPro software (Molecular Devices). HaCaT were seeded with or without HDM extract (25 µg/mL) and then treated with culture supernatants from T cells, T cells treated with human IL-22 antibody (R&D), or T cells treated with HDM extract. The supernatants were harvested after 24, 48, or 72 h, respectively, and the concentrations of TARC, IL-1α, and IL-6 in the culture supernatants were assessed by ELISA according to the manufacturer's instructions (Biolegend, San Diego, CA, USA). Relative absorbance was measured at 450 nm, and concentration was calculated using SpectraMac iD3 (Molecular Devices).

2.8. Transwell migration assay

Transwell migration assay was performed using a microchamber Transwell system with 5 µm pores (Corning Costar, Tewksbury, MA, USA). Human T cells in RPMI1640 medium supplemented with 1% heat-inactivated FBS were added to each upper compartment of the insert, and culture supernatants from HaCaT (48 h and 72 h) treated with HDM extract (25 µg/mL) or HDM extract (25 µg/mL) were added to each lower compartment. T cells were allowed to migrate to the lower compartment for 3 h. The upper compartment was removed, and T cells in the lower compartment were evaluated and expressed as a percentage of the total number of T cells added to the upper well.

2.9. Gene expression profiling

Gene expression of HaCaT treated with HDM, VED, ALTamer, or ALOVEtamer was analyzed using Affymetrix GeneChip® Human Gene 2.0 ST arrays. Total cellular RNA was extracted from 1×10^6 cells using TRIzol (Invitrogen). RNA quality was assessed by an Agilent 2100 Bioanalyzer using an RNA 6000 Nano Chip (Agilent Technologies, Santa Clara, CA, USA), and the quantity was determined using a Nanodrop-1000 Spectrophotometer (Thermo Scientific). An aliquot of 300 ng of each RNA sample was used for the Affymetrix procedure as recommended by the manufacturer. DEGs were functionally annotated using a web-based tool, the Database for Annotation, Visualization, and Integrated Discovery (DAVID), and classified based on information about gene function, including basic statistics, hierarchical clustering, K-means, *t*-test, and analysis of variance (ANOVA). An integrated analysis (mRNA/miRNA) database was analyzed to identify regulatory networks.

2.10. Statistical Analysis

Data were expressed as mean $\pm$ SD and compared between two groups using an unpaired two-tailed *t*-test. Comparisons between three or more groups were performed using one-way ANOVA followed by the Newman–Keuls multiple comparison test. A *p*-value < 0.05 was considered statistically significant. All statistical tests were performed using Graph-Pad InStat (GraphPad Software, San Diego, CA, USA).

III. Results

3.1. ALOVEtamer inhibits HDM-induced proliferation of HaCaT and primary human keratinocytes

Keratinocyte proliferation is closely related to the inflammatory response in skin diseases, including psoriasis and atopic diseases [16,34]. In addition, HDM extract induces skin inflammation and proliferation of HaCaT skin keratinocyte cell lines. Therefore, we evaluated whether ALOVEtamer inhibits HDM-induced proliferation of HaCaT and primary human keratinocytes. We found that both VED and ALOVEtamer effectively inhibited HDM extract-induced proliferation of HaCaT and human primary keratinocytes (Fig. S1). ALOVEtamer showed a stronger effect than VED.

3.2. ALOVEtamer inhibits HDM-induced IL-1 α and IL-6 production by HaCaT and primary keratinocytes

HDM extract also increases IL-1 α and IL-6 production by HaCaT. Therefore, we evaluated whether ALOVEtamer could inhibit HDM-induced IL-1 α and IL-6 production by HaCaT and primary keratinocytes. IL-1 α and IL-6 production by HaCaT and primary keratinocytes was significantly increased by treatment with HDM extract, but this production was effectively inhibited by treatment with VED, ALTamer, and ALOVEtamer (Figure 1).

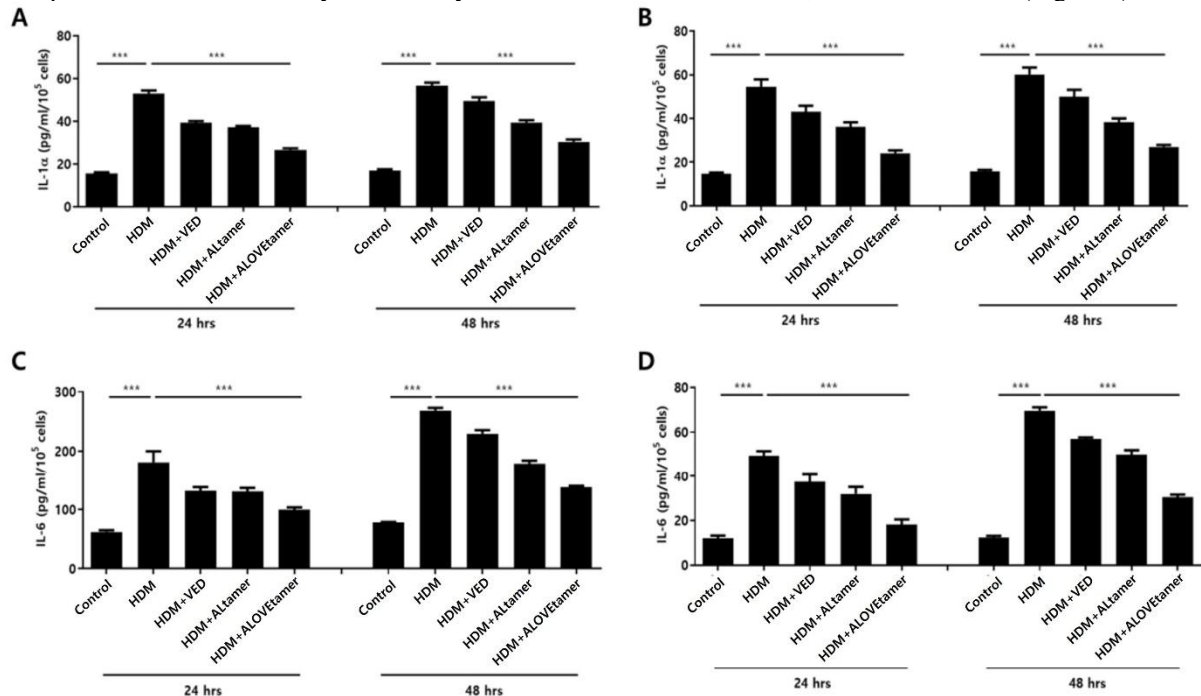


Figure 1. Effect of HDM extract on IL-1 α and IL-6 production by HaCaT and primary keratinocytes and their inhibition by ALOVEtamer. HaCaT (A, C) and primary human keratinocytes (B, D) were treated with 25 μ g/mL HDM extract with or without VED (40 μ M), ALTamer (0.09 μ g/mL), or ALOVEtamer (VED 40 μ M/ALTamer 0.09 μ g/mL) for 24 or 48 h. Culture supernatants were collected, and the concentrations of IL-1 α (A, B) and IL-6 (C, D) were assessed by ELISA as described in Materials and Methods. Data are expressed as mean $\pm$ SD. *** *p* < 0.001 .

3.3. ALOVEtamer inhibits HDM-induced IL-17 and IL-22 production by T cells

IL-17 and IL-22 are closely related to the pathogenesis of skin inflammation, especially atopic diseases [7,35,36]. Therefore, we investigated the ability of ALOVEtamer to modulate the expression of HDM-induced IL-17 and IL-22 production by human peripheral T cells. T cells purified from human PBMCs were exposed to HDM extract in the presence or absence of VED, ALTamer, or ALOVEtamer, and the results showed that HDM extract increased IL-17 and IL-22 production, which was effectively suppressed by ALOVEtamer (Fig. 2).

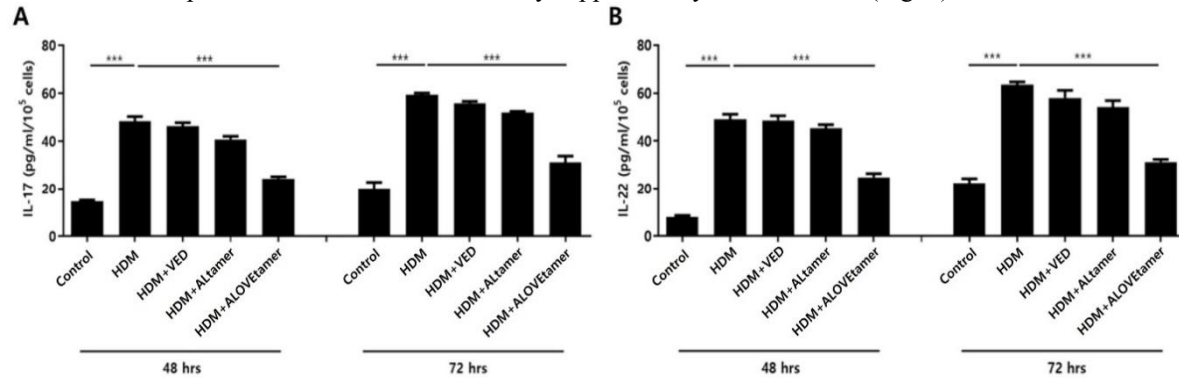


Figure 2. Effect of HDM extract on IL-17 and IL-22 production by T cells and its inhibition by ALOVEtamer. T cells isolated from peripheral blood mononuclear cells (PBMCs) and purified by the MACS negative selection system were treated with 25 μ g/mL of HDM extract with or without VED (40 μ M), ALTamer (0.09 μ g/mL), or ALOVEtamer (VED 40 μ M/ALTamer 0.09 μ g/mL) for 48 or 72 h as described in Materials and Methods. Culture supernatants were collected, and the concentrations of IL-17 (A) and IL-22 (B) were measured by ELISA. Data are expressed as mean $\pm$ SD. *** $p < 0.001$.

3.4. ALOVEtamer inhibits HDM-induced IL-22R α expression by HaCaT and primary keratinocytes

Because IL-22R α plays a role in HDM-induced skin inflammation, we evaluated whether ALOVEtamer could modulate HDM-induced IL-22R α expression by HaCaT and primary keratinocytes. Immunofluorescence staining showed that HDM extract significantly increased IL-22R α expression in HaCaT and primary keratinocytes, but this increase was inhibited by treatment with ALOVEtamer (Figure 3). Interestingly, VED could not modulate IL-22R α expression, whereas ALTamer could.

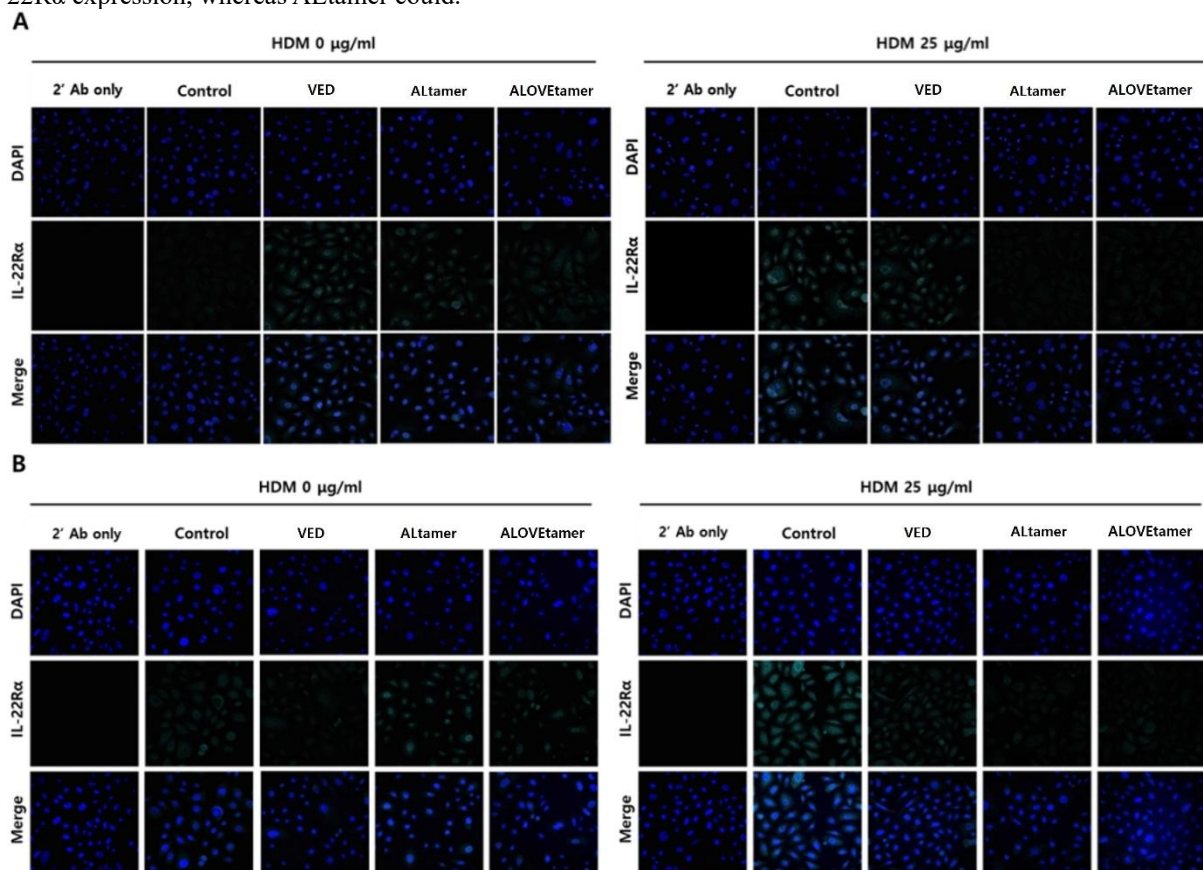


Figure 3. Effect of HDM extract on IL-22R α expression by HaCaT and primary keratinocytes and inhibition by ALOVEtamer. (A) HaCaT and (B) primary keratinocytes were pretreated with HDM (25 μ g/mL) for 24 h, fixed with 4% paraformaldehyde (PFA), and preincubated with 5% goat serum in PBS-T. Cells were incubated with anti-human IL-22R α antibody (1:150) or vehicle plus Alexa Fluor 633-conjugated anti-rabbit secondary antibody (1:1500), followed by nuclear staining with 4',6-diamidino-2-phenylindole (DAPI; blue). Fluorescence microscopy images were captured and analyzed by confocal microscopy. Results are the representative values of three independent experiments.

3.5. ALOVEtamer inhibits TARC production by HaCaT and primary keratinocytes and suppresses T cell migration

TARC is involved in the chemotaxis of T cells, especially Th2 cells, and promotes T cell migration to skin lesions, thereby contributing to the pathogenesis of skin inflammation in atopic dermatitis [4,37]. In addition, TARC production results from the interaction between IL-22 and its receptor on skin keratinocytes [6,12]. Since ALOVEtamer down-regulates HDM-induced IL-22 production by T cells and IL-22R α expression by HaCaT, we evaluated the effect of ALOVEtamer on TARC production. As expected, HDM extract increased TARC production by HaCaT, and this increase was effectively down-regulated by ALOVEtamer treatment (Fig. 4A). This decrease in TARC production was accompanied by a decrease in T cell migration (Fig. 4B).

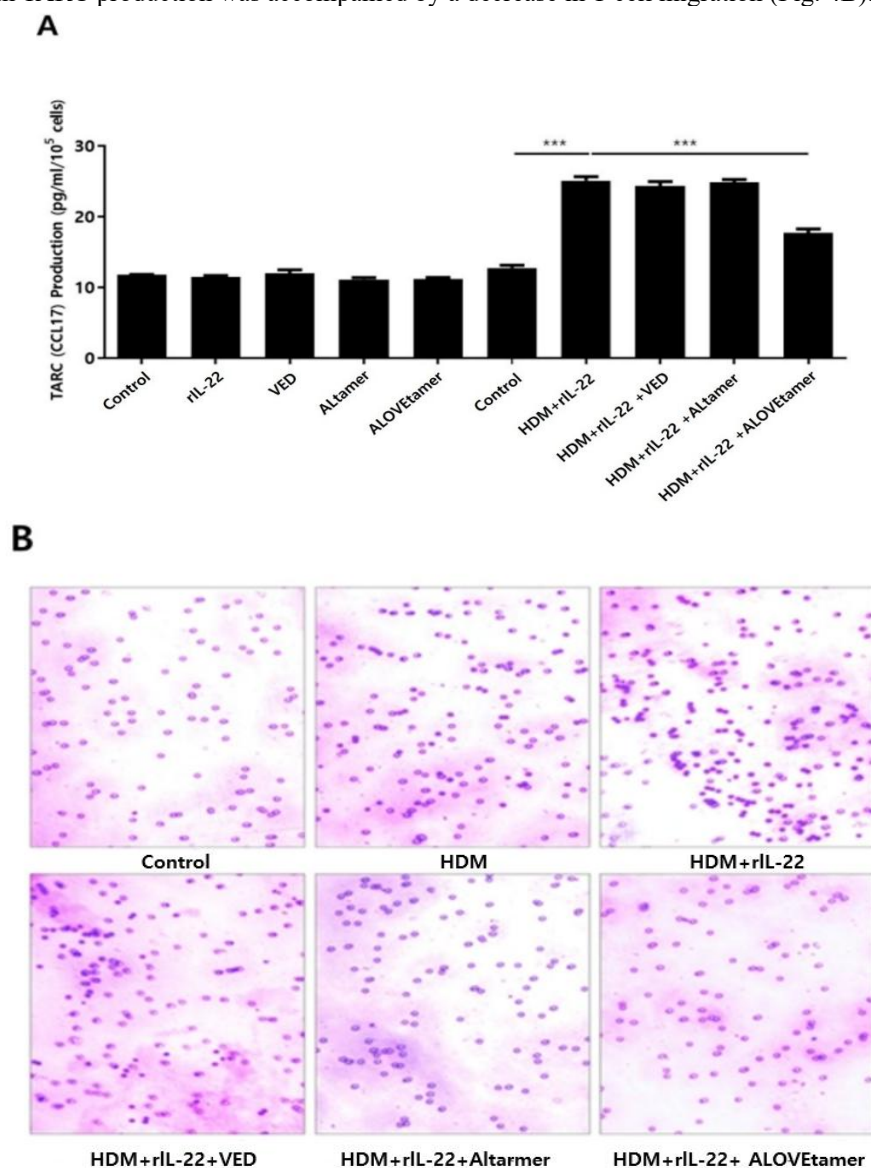


Figure 4. Effects of HDM extract on thymic and activation-regulating chemokine (TARC) production and T cell migration induced by HaCaT, and inhibition of these effects by ALOVEtamer. (A) ALOVEtamer down-regulates HDM extract-associated increase in TARC production induced by HaCaT. Data are presented as mean $\pm$ SD. *** $p < 0.001$. (B) Transwell migration assays showing ALOVEtamer-associated inhibition of HDM extract-enhanced T cell migration for 48 or 72 h. Results are representative of three independent experiments.

3.6. ALOVEtamer Suppresses HDM-Induced GDNF Production by HaCaT and Primary Keratinocytes

Factors altered in HaCaT and primary keratinocytes by treatment with HDM extract and ALOVEtamer were analyzed using the Human Gene 2.0 ST array. The expression of 31 genes was increased by treatment with HDM extract, but decreased by treatment with ALOVEtamer (Supplementary Table S1). Among these changes were alterations in the expression of mRNA-encoding glial cell line-derived neurotrophic factor (GDNF), the major causative factor for itching during skin inflammation in atopic diseases [24,38]. To assess whether HDM extract and ALOVEtamer induced similar changes in GDNF protein expression, the concentrations of GDNF protein in cell supernatants were assayed by ELISA. Treatment of HaCaT and primary keratinocytes with HDM extract markedly increased GDNF production, an increase effectively suppressed by treatment with ALOVEtamer (Figure 5).

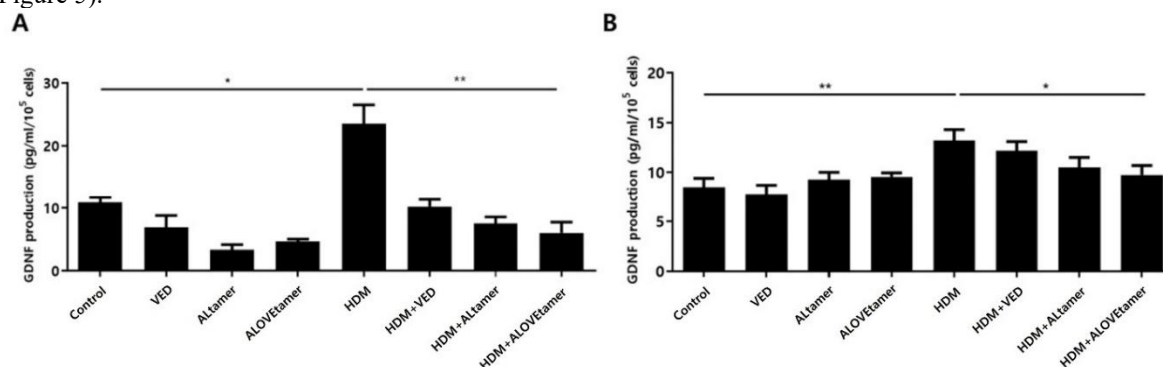


Figure 5. Effect of HDM extract on GDNF production by HaCaT and primary keratinocytes and inhibition by ALOVEtamer. HaCaT (A) and primary human keratinocytes (B) were treated with 25 $\mu\text{g/mL}$ HDM extract with or without VED, ALTamer, or ALOVEtamer for 48 h. Culture supernatants were collected, and GDNF concentrations were measured by ELISA. Data are expressed as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$.

IV. Discussion

Atopic dermatitis (AD) is a common chronic inflammatory skin disease characterized by pruritus, erythema, and eczema. The etiology of AD is highly diverse and may include both genetic and environmental factors [39,40]. HDM is an environmental factor frequently associated with the development of AD, but few studies have analyzed substances that modulate the development of HDM-related AD to date [4,11,41]. Previous studies have shown that treatment with HDM extract increases IL-22R α expression and TARC production by T cells, and enhances IL-22 production and migration by T cells [4,42,43]. In this study, we extend these results by analyzing substances that can modulate HDM-induced inflammatory responses in skin keratinocytes and T cells.

ALOVEtamer is a compound that combines VED and an aptamer, a DNA fragment that specifically binds to VED. ALOVEtamer-specific aptamers enhance the stability of VED by preventing rapid oxidation of VED through interaction with oxygen or aqueous solutions [44]. Moreover, VED increases skin hydration and improves itching [30]. It also showed neuroprotective effects in an animal model of Parkinson's disease induced by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [23]. The onset and progression of skin diseases, including atopic dermatitis and psoriasis, are closely related to ROS-mediated immune responses, and VED, an antioxidant, is an effective anti-inflammatory molecule because it acts as a ROS scavenger [45]. Therefore, ALOVEtamer may be a promising candidate for regulating HDM-induced skin inflammation and treating atopic dermatitis.

Inflammatory responses in the skin are associated with proliferation of keratinocytes and are therefore induced by HDM extracts [4,46–48]. Therefore, substances that regulate keratinocyte proliferation induced by HDM extracts may also be useful for regulating inflammation in skin diseases such as atopic dermatitis. As expected, ALOVEtamer effectively regulates proliferation of HaCaT skin keratinocyte cell lines and primary skin keratinocytes induced by HDM extracts. Since psoriasis is caused by proliferation of keratinocytes, ALOVEtamer may be an effective treatment. To confirm this, studies are in progress to investigate its effects in psoriasis animal models including imiquimod-induced psoriasis and 3D skin models based on primary human keratinocytes.

Keratinocytes play an important role in forming the skin barrier and inducing skin immune responses by releasing various cytokines (IL-1 α , IL-6, etc.), and thus are involved in innate immunity [28,49]. When keratinocytes induce excessive inflammatory cytokines (such as IL-1 α and IL-6) mediated by HDM extract, a severe inflammatory response may occur in the skin [4,11,28,49]. Therefore, inhibition of keratinocyte proliferation mediated by ALOVEtamer not only controls the inflammatory response of the skin by suppressing the increased production of IL-1 α and IL-6, but also inhibits keratinocytes from directly producing these cytokines.

IL-22 is involved in the induction of keratinocyte migration and proinflammatory gene expression, as well as in the induction of proliferation of normal human epidermal keratinocytes [28,50,51]. IL-22 production in

skin lesions of atopic dermatitis animal models and atopic dermatitis patients is higher than that of normal controls, respectively [41,49,52,53]. Furthermore, IL-6 production by peripheral blood T cells is higher in atopic dermatitis patients than in controls [54]. Treatment of keratinocytes with HDM extract and rIL-22 increases IL-6 production. IL-22 is produced by CD3-c-Kit⁺ cells in skin lesions of patients with psoriasis and atopic dermatitis, and c-Kit⁺ FcεRI⁺ mast cells are the major cellular source of IL-22 in atopic dermatitis patients [53]. Downregulation of IL-22Rα expression may control the inflammatory process in skin lesions of psoriasis and atopic dermatitis patients. IL-22R consists of IL-22Rα and IL-10Rβ, and IL-22Rα expression is particularly increased, which is considered a hallmark of inflammation in the skin [26,51]. HDM extract increases IL-22Rα expression in keratinocytes [4,11,46]. Moreover, IL-22Rα interacts with IL-22 and is closely involved in the inflammatory process of the skin, and the expression of IL-22 by T cells is enhanced by HDM extract (4). The increased IL-22Rα expression in both HaCaT cells and primary keratinocytes was suppressed by ALOVEtamer treatment. This strongly suggests that ALOVEtamer effectively regulates IL-22-mediated skin inflammation not only by suppressing IL-22 production by T cells but also by suppressing IL-22Rα expression in keratinocytes.

In addition to its involvement in the production of proinflammatory cytokines, the interaction between IL-22 and IL-22Rα plays an important role in T cell migration by increasing the production of TARC, a chemokine that plays a key role in the pathogenesis of AD. TARC production is higher in patients with atopic dermatitis and the AD animal model NC/Nga mice than in their normal controls. Keratinocytes are the major cellular source of TARC, and TARC production in these cells is increased by HDM extract [4,5,42,55]. TARC promotes T cell migration [43,56,57]. In this study, we found that ALOVEtamer inhibited the increase in TARC expression induced by HDM extract and rIL-22 treatment. ALOVEtamer also effectively inhibited the T cell migration induced by the culture supernatant of HaCaT treated with HDM extract in the presence of rIL-22. Thus, Aptamin C inhibits HDM-induced IL-22 production by T cells while simultaneously inhibiting the migration of IL-22-producing T cells to skin lesions.

Molecular analysis of the effect of HDM with or without ALOVEtamer on gene expression in HaCaT and primary keratinocytes identified 31 genes, the expression of which was significantly increased by treatment with HDM extract but significantly decreased by treatment with ALOVEtamer. One of these genes encodes GDNF, which was originally identified to enhance neuronal survival via increased dopamine uptake but has recently been reported to be closely related to inflammation, particularly severe pruritus in atopic dermatitis [38]. In addition, artemin, a member of the GDNF family, has been implicated in hypersensitivity to warm sensation, similar to the warmth-induced pruritus in atopic dermatitis [58]. Therefore, we evaluated whether HDM extract could increase GDNF production by HaCaT and primary keratinocytes and whether this increase could be inhibited by ALOVEtamer. As expected, HDM extract significantly increased GDNF protein production, and this increase was suppressed by treatment with ALOVEtamer. Cream containing ALOVEtamer has a skin moisturizing effect and reduces itching in normal people with dry skin. Ongoing clinical studies have shown that ointment containing ALOVEtamer effectively controls itching in patients with atopic dermatitis. This study reports the anti-inflammatory effect of ALOVEtamer on skin inflammation and its related mechanism. The results of the study suggest that ALOVEtamer may be a candidate for the treatment of skin inflammation, especially itching. Since ALOVEtamer increases the stability of VED, ALOVEtamer may further improve diseases effectively treated with VED.

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References

- [1]. Ong, P.Y. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N. Engl. J. Med.* 2002, 347, 1151–1160.
- [2]. Boguniewicz, M.; Leung, D.Y. Recent insights into atopic dermatitis and implications for management of infectious complications. *J. Allergy Clin. Immunol.* 2010, 125, 4–13.
- [3]. Lee, C.-H.; Yu, H.-S. Biomarkers for Itch and Disease Severity in Atopic Dermatitis. *Pathog. Manag. Atopic Dermat.* 2011, 41, 136–148.
- [4]. Jang, M.; Kim, H.; Kim, Y.; Choi, J.; Jeon, J.; Hwang, Y.; Kang, J.S.; Lee, W.J. The crucial role of IL-22 and its receptor in thymus and activation regulated chemokine production and T-cell migration by house dust mite extract. *Exp. Dermatol.* 2016, 25, 598–603.
- [5]. Taube, C.; Tertilt, C.; Gyulveszi, G.; Dehzad, N.; Kreyemborg, K.; Schneeweiss, K.; Michel, E.; Reuter, S.; Renauld, J.-C.; Arnold-Schild, D.; et al. IL-22 Is Produced by Innate Lymphoid Cells and Limits Inflammation in Allergic Airway Disease. *PLoS ONE* 2011, 6, e21799.
- [6]. Kunkl, M.; Amornino, C.; Frascolla, S.; Sambucci, M.; De Bardi, M.; Caristi, S.; Arcieri, S.; Battistini, L.; Tuosto, L. CD28 Autonomous Signaling Orchestrates IL-22 Expression and IL-22-Regulated Epithelial Barrier Functions in Human T Lymphocytes. *Front. Immunol.* 2020, 11, 590964.
- [7]. Sanjabi, S.; Zenewicz, L.A.; Kamanaka, M.; Flavell, R.A. Anti-inflammatory and pro-inflammatory roles of TGF-β, IL-10, and IL-

- 22 in immunity and autoimmunity. *Curr. Opin. Pharmacol.* 2009, 9, 447–453.
- [8]. Furue, M. Regulation of Filaggrin, Loricrin, and Involucrin by IL-4, IL-13, IL-17A, IL-22, AHR, and NRF2: Pathogenic Implications in Atopic Dermatitis. *Int. J. Mol. Sci.* 2020, 21, 5382.
- [9]. Sharma, D.K.; Ashawat, M.S. Pathophysiology and Management of Atopic Dermatitis: A Laconic Review. *Curr. Drug Ther.* 2020, 15, 321–336.
- [10]. Kim, M.H.; Jin, S.-P.; Jang, S.; Choi, J.-Y.; Chung, D.H.; Lee, D.H.; Kim, K.H.; Kim, H.Y. IL-17A–Producing Innate Lymphoid Cells Promote Skin Inflammation by Inducing IL-33–Driven Type 2 Immune Responses. *J. Investig. Dermatol.* 2020, 140, 827–837.e9.
- [11]. Kim, Y.-H.; Kang, M.; Kim, T.; Jeong, Y.; Ahn, J.-O.; Choi, J.; Chung, J.-Y. Anti-Inflammatory and Immune Modulatory Effects of Synbio-Glucan in an Atopic Dermatitis Mouse Model. *Nutrients* 2021, 13, 1090.
- [12]. Gendrisch, F.; Esser, P.R.; Schempp, C.M.; Wölflle, U. Luteolin as a modulator of skin aging and inflammation. *BioFactors* 2021, 47, 170–180.
- [13]. Um, J.-Y.; Kim, H.B.; Kang, S.Y.; Son, J.H.; Chung, B.Y.; Park, C.W.; Kim, H.O. 2,3,7,8-Tetrachlorodibenzo-p-Dioxin Regulates the Expression of Aryl Hydrocarbon Receptor-Related Factors and Cytokines in Peripheral Blood Mononuclear Cells and CD4⁺ T cells from Patients with Atopic Dermatitis and Psoriasis. *Ann. Dermatol.* 2020, 32, 360–369.
- [14]. Wang, B.; Han, D.; Li, F.; Hou, W.; Wang, L.; Meng, L.; Mou, K.; Lu, S.; Zhu, W.; Zhou, Y. Elevated IL-22 in psoriasis plays an anti-apoptotic role in keratinocytes through mediating Bcl-xL/Bax. *Apoptosis* 2020, 25, 663–673.
- [15]. Gonçalves, R.S.G.; Pereira, M.C.; Dantas, A.T.; Almeida, A.; Marques, C.D.L.; Rego, M.J.B.M.; Pitta, I.R.; Duarte, A.L.B.P.; Pitta, M.G.R. IL-17 and related cytokines involved in systemic sclerosis: Perspectives. *Autoimmunity* 2017, 51, 1–9.
- [16]. Deng, Y.; Chang, C.; Lu, Q. The Inflammatory Response in Psoriasis: A Comprehensive Review. *Clin. Rev. Allergy Immunol.* 2016, 50, 377–389.
- [17]. Wahlgren, C.-F. Itch and Atopic Dermatitis: An Overview. *J. Dermatol.* 1999, 26, 770–779.
- [18]. Darsow, U.; Scharein, E.; Bromm, B.; Ring, J. Skin testing of the pruritogenic activity of histamine and cytokines (interleukin-2 and tumour necrosis factor- α) at the dermal–epidermal junction. *Br. J. Dermatol.* 1997, 137, 415–417.
- [19]. Pincelli, C.; Fantini, F.; Massimi, P.; Girolomoni, G.; Seidenari, S.; Giannetti, A. Neuropeptides in skin from patients with atopic dermatitis: An immunohistochemical study. *Br. J. Dermatol.* 1990, 122, 745–750.
- [20]. Scholzen, T.; Armstrong, C.A.; Bunnett, N.W.; Luger, T.A.; Olerud, J.E.; Ansel, J.C. Neuropeptides in the skin: Interactions between the neuroendocrine and the skin immune systems. *Exp. Dermatol.* 1998, 7, 81–96.
- [21]. Akane, T.; Hiroshi, M. Expression of nerve growth factor in itchy skins of atopic NC/NgaTnd mice. *J. Vet. Med. Sci.* 2005, 67, 915–919.
- [22]. Tobin, D.; Nabarro, G.; De La Faute, H.B.; Van Vloten, W.A.; Van Der Putte, S.C.; Schuurman, H.-J. Increased number of immunoreactive nerve fibers in atopic dermatitis. *J. Allergy Clin. Immunol.* 1992, 90, 613–622.
- [23]. Zhang, S.; Ma, Y.; Feng, J. Neuroprotective mechanisms of α -viniferin in a rotenone-induced cell model of Parkinson's disease: significance of SIRT3-mediated FOXO3 deacetylation. *Neural Regen Res.* 2020, 15(11), 2143–2153.
- [24]. Tokime, K.; Katoh-Semba, R.; Yamanaka, K.; Mizoguchi, A.; Mizutani, H. Enhanced production and secretion of glial cell line-derived neurotrophic factor and nerve growth factor from the skin in atopic dermatitis mouse model. *Arch. Dermatol. Res.* 2008, 300, 343–352.
- [25]. Kohen, R. Skin antioxidants: Their role in aging and in oxidative stress—New approaches for their evaluation. *Biomed. Pharmacother.* 1999, 53, 181–192.
- [26]. Cho, K.A.; Suh, J.W.; Lee, K.H.; Kang, J.L.; Woo, S.Y. IL-17 and IL-22 enhance skin inflammation by stimulating the secretion of IL-1 β by keratinocytes via the ROS-NLRP3-caspase-1 pathway. *Int. Immunol.* 2012, 24, 147–158.
- [27]. Li, C.-W.; Li, L.-L.; Chen, S.; Zhang, J.-X.; Lu, W.-L. Antioxidant Nanotherapies for the Treatment of Inflammatory Diseases. *Front. Bioeng. Biotechnol.* 2020, 8, 200.
- [28]. Kim, D.M.; Park, H.L.; Jung, G.H.; Lee, B.H.; Kim, K.L.; Nam, J.K. The anti-inflammatory effect of aloe on UVB-induced skin inflammation through the down-regulation of pro-inflammatory cytokines. *Antimicrob. Res.* 2023, 212, 214–226.
- [29]. Kim, D.M.; Yun, G.J.; Park, I.M.; Gan, S.H.; Fuloria, F.S.; Sekar, M.W.; Khattulanuar, S.F. Inhibition of Aloe exosome oxidation by PDRN aptamers. *Molecules Res.* 2020, 5, 17–29.
- [30]. Kim, D.M.; Yun, G.J.; Park, I.M.; Gan, S.H.; Fuloria, F.S.; Sekar, M.W.; Khattulanuar, S.F.; Rani, N.N. Viniferin improves hyperpigmentation via accelerating feedback termination of cAMP/PKA in facultative melanogenesis. *Immuno. J.* 2022, 12(5), 145–157.
- [31]. Kim, D.M.; Yun, G.J.; Park, I.M.; Zhang, S.; Ma, Y.; Feng, J. The analysis of citric acid concentration in organs of gulo(-/-) mice upon vitamin C withdrawal. *J. Bio. Nutri.* 2022, 18, 22–32.
- [32]. Kim, H.; Kim, Y.; Bae, S.; Lim, S.H.; Jang, M.; Choi, J.; Jeon, J.; Hwang, Y.; Kang, J.S.; Lee, W.J. Vitamin C Deficiency Causes Severe Defects in the Development of the Neonatal Cerebellum and in the Motor Behaviors of Gulo(-/-) Mice. *Antioxid. Redox Signal.* 2015, 23, 1270–1283.
- [33]. Kim, A.R.; Kim, S.H.; Kim, D.; Cho, S.W.; Son, A.; Yoon, M.-Y. Detection of Nonylphenol with a Gold-Nanoparticle-Based Small-Molecule Sensing System Using an ssDNA Aptamer. *Int. J. Mol. Sci.* 2019, 21, 208.
- [34]. Cibrian, D.; de la Fuente, H.; Sánchez-Madrid, F. Metabolic Pathways That Control Skin Homeostasis and Inflammation. *Trends Mol. Med.* 2020, 26, 975–986.
- [35]. Bernardini, N.; Skroza, N.; Tolino, E.; Mambrin, A.; Anzalone, A.; Balduzzi, V.; Colapietra, D.; Marchesiello, A.; Michelini, S.; Proietti, I.; et al. IL-17 and its role in inflammatory, autoimmune, and oncological skin diseases: State of art. *Int. J. Dermatol.* 2019, 59, 406–411.
- [36]. Sugaya, M. The Role of Th17-Related Cytokines in Atopic Dermatitis. *Int. J. Mol. Sci.* 2020, 21, 1314.
- [37]. Choi, J.H.; Lee, G.H.; Jin, S.W.; Kim, J.Y.; Hwang, Y.P.; Han, E.H.; Kim, Y.H.; Jeong, H.G. Impressive Acid Ameliorates Atopic Dermatitis-Like Skin Lesions by Inhibiting ERK1/2-Mediated Phosphorylation of NF- κ B and STAT1. *Int. J. Mol. Sci.* 2021, 22, 2334.
- [38]. Morel, L.; Domingues, O.; Zimmer, J.; Michel, T. Revisiting the Role of Neurotrophic Factors in Inflammation. *Cells* 2020, 9, 865.
- [39]. Ahn, K.; Kim, B.E.; Kim, J.; Leung, D.Y. Recent advances in atopic dermatitis. *Curr. Opin. Immunol.* 2020, 66, 14–21.
- [40]. Nakatsuji, T.; Hata, T.R.; Tong, Y.; Cheng, J.Y.; Shafiq, F.; Butcher, A.M.; Salem, S.S.; Brinton, S.L.; Spergel, A.K.R.; Johnson, K.; et al. Development of a human skin commensal microbe for bacteriotherapy of atopic dermatitis and use in a phase 1 randomized clinical trial. *Nat. Med.* 2021, 27, 700–709.
- [41]. Lim, H.-S.; Ha, H.; Lee, M.-Y.; Jin, S.-E.; Jeong, S.-J.; Jeon, W.-Y.; Shin, N.-R.; Sok, D.-E.; Shin, H.-K. Saussurea lappa alleviates inflammatory chemokine production in HaCaT cells and house dust mite-induced atopic-like dermatitis in Nc/Nga mice. *Food Chem. Toxicol.* 2014, 63, 212–220.
- [42]. Saeki, H.; Tamaki, K. Thymus and activation regulated chemokine (TARC)/CCL17 and skin diseases. *J. Dermatol. Sci.* 2006, 43, 75–84.

- [43]. Vestergaard, C.; Bang, K.; Gesser, B.; Yoneyama, H.; Matsushima, K.; Larsen, C.G. A Th2 Chemokine, TARC, Produced by Keratinocytes May Recruit CLA+CCR4+ Lymphocytes into Lesional Atopic Dermatitis Skin. *J. Investig. Dermatol.* 2000, 115, 640–646.
- [44]. Kim, D.M.; Yun, G.J.; Park, I.M.; Gan, S.H.; Fuloria, F.S.; Sekar, M.W.; Khattulanuar, S.F.; Rani, N.N. Vitamin C replacement chemistry, biosynthesis and pharmacology of Viniferin: Potential resveratrol-derived molecules for new drug discovery, development and therapy. *Molecules.* 2022, 27, 5072–2081.
- [45]. Leveque, N.; Robin, S.; Muret, P.; Mac-Mary, S.; Makki, S.; Humbert, P. High iron and low ascorbic acid concentrations in the dermis of atopic dermatitis patients. *Dermatology* 2003, 207, 261–264.
- [46]. Choi, D.-I.; Park, J.-H.; Choi, J.-Y.; Piao, M.; Suh, M.-S.; Lee, J.-B.; Yun, S.-J.; Lee, S.-C. Keratinocytes-Derived Reactive Oxygen Species Play an Active Role to Induce Type 2 Inflammation of the Skin: A Pathogenic Role of Reactive Oxygen Species at the Early Phase of Atopic Dermatitis. *Ann. Dermatol.* 2021, 33, 26–36.
- [47]. Yamanishi, Y.; Mogi, K.; Takahashi, K.; Miyake, K.; Yoshikawa, S.; Karasuyama, H. Skin-infiltrating basophils promote atopic dermatitis-like inflammation via IL-4 production in mice. *Allergy* 2020, 75, 2613–2622.
- [48]. Nakahara, T.; Kido-Nakahara, M.; Tsuji, G.; Furue, M. Basics and recent advances in the pathophysiology of atopic dermatitis. *J. Dermatol.* 2021, 48, 130–139.
- [49]. Guilleoteau, K.; Paris, I.; Pedretti, N.; Boniface, K.; Juchaux, F.; Huguier, V.; Guillet, G.; Bernard, F.-X.; Lecron, J.-C.; Morel, F. Skin Inflammation Induced by the Synergistic Action of IL-17A, IL-22, Oncostatin M, IL-1 α , and TNF- α Recapitulates Some Features of Psoriasis. *J. Immunol.* 2010, 184, 5263–5270.
- [50]. Boniface, K.; Bernard, F.-X.; Garcia, M.; Gurney, A.L.; Lecron, J.-C.; Morel, F. IL-22 Inhibits Epidermal Differentiation and Induces Proinflammatory Gene Expression and Migration of Human Keratinocytes. *J. Immunol.* 2005, 174, 3695–3702. [CrossRef] [PubMed]
- [51]. Mitra, A.; Raychaudhuri, S.K.; Raychaudhuri, S.P. IL-22 induced cell proliferation is regulated by PI3K/Akt/mTOR signaling cascade. *Cytokine* 2012, 60, 38–42.
- [52]. Fujita, H. The role of IL-22 and Th22 cells in human skin diseases. *J. Dermatol. Sci.* 2013, 72, 3–8.
- [53]. Mashiko, S.; Bouguermouh, S.; Rubio, M.; Baba, N.; Bissonnette, R.; Sarfati, M. Human mast cells are major IL-22 producers in patients with psoriasis and atopic dermatitis. *J. Allergy Clin. Immunol.* 2015, 136, 351–359.e1.
- [54]. Toshitani, A.; Ansel, J.C.; Chan, S.C.; Li, S.-H.; Hanifin, J.M. Increased Interleukin 6 Production by T Cells Derived from Patients with Atopic Dermatitis. *J. Investig. Dermatol.* 1993, 100, 299–304.
- [55]. Morita, E.; Takahashi, H.; Niihara, H.; Dekio, I.; Sumikawa, Y.; Murakami, Y.; Matsunaka, H. Stratum corneum TARC level is a new indicator of lesional skin inflammation in atopic dermatitis. *Allergy* 2010, 65, 1166–1172.
- [56]. Imai, T.; Baba, M.; Nishimura, M.; Kakizaki, M.; Takagi, S.; Yoshie, O. The T Cell-directed CC Chemokine TARC Is a Highly Specific Biological Ligand for CC Chemokine Receptor 4. *J. Biol. Chem.* 1997, 272, 15036–15042.
- [57]. Campbell, J.J.; Haraldsen, G.; Pan, J.; Rottman, J.; Qin, S.; Ponath, P.; Andrew, D.P.; Warnke, R.; Ruffing, N.; Kassam, N.; et al. The chemokine receptor CCR4 in vascular recognition by cutaneous but not intestinal memory T cells. *Nature* 1999, 400, 776–780.
- [58]. Murota, H.; Izumi, M.; El-Latif, M.I.A.; Nishioka, M.; Terao, M.; Tani, M.; Matsui, S.; Sano, S.; Katayama, I. Artemin causes hypersensitivity to warm sensation, mimicking warmth-provoked pruritus in atopic dermatitis. *J. Allergy Clin. Immunol.* 2012, 130, 671–682.e4.