Therapeutic potential of a systemically applied humanized monoclonal antibody targeting Toll-like receptor 2 in atopic-dermatitis-like skin lesions in a mouse model

HYUNJIN YEO[1], EUITAEK JUNG[1], TAE YOON KIM[1] and SOON YOUNG SHIN[1,2]

[1]Department of Biological Sciences, Sanghuh College of Life Science, Konkuk University, Seoul 05029, Republic of Korea; [2]Cancer and Metabolism Institute, Konkuk University, Seoul 05029, Republic of Korea

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Abstract. Atopic dermatitis (AD) is a prevalent, persistent inflammatory skin disorder distinguished by pruritic and irritated skin. Toll-like receptors (TLRs) are specialized receptors that recognize specific patterns associated with pathogens and tissue damage, triggering an innate immune response that protects the host from invading pathogens. Previously, it was demonstrated that intradermal injection of the humanized anti-TLR2 monoclonal antibody (Ab) Tomaralimab effectively relieved AD-like skin inflammation in BALB/c mouse models exposed to house dust mite extracts. However, it remains unclear whether allergenic hapten-induced AD can be effectively treated with systemically administered TLR2-targeting Abs. In the present study, it was observed that administrating Tomaralimab through intravenous injection alleviated AD-like skin lesions in BALB/c mice challenged with topical application of 2,4-dinitrochlorobenzene by reducing the infiltration of inflammatory cells into skin lesions and preventing the creation of various inflammatory cytokines, including thymic stromal lymphopoietin, interleukin (IL)-4, IL-13, IL-17 and IL-31, which are associated with the pathogenesis of AD. These findings support the feasibility of using a humanized anti-TLR2 monoclonal Ab as systemic therapy for AD.

Introduction

Atopic dermatitis (AD) is a common chronic inflammatory skin disease characterized by dry, itchy and inflamed skin (1). Although AD commonly develops during childhood, it can develop at any age. The precise cause of AD is not fully understood; however, it is considered to result from a complex interplay between genetic, environmental and dysregulated immune systems (1). Various systemic and topical medications, including immunosuppressants, Janus kinase (JAK) inhibitors, and therapeutic monoclonal antibodies (Abs), have been developed for the treatment of AD (2-4). However, potentially unwanted side effects limit their long-term applicability (5,6). Given the multifactorial aspects of the pathogenesis of AD, the identification of additional molecular targets with minimal side effects is required for optimal AD treatment.

Toll-like receptors (TLRs) are pattern recognition receptors primarily responsible for recognizing microbial pathogen-associated molecular patterns (PAMPs), thus initiating an innate immune response to defend the host against invading pathogens. TLRs also recognize internal danger signals known as damage-associated molecular patterns (DAMPs), which are released by cells that are activated or damaged during the inflammatory process (7). The activation of TLR pathways plays a role in the development of AD (8). Among the TLR members, TLR2 is involved in sensing a broad range of PAMPs (9).

Tomaralimab is a novel humanized monoclonal Ab targeting TLR2 (10). The authors' previous findings showed that intradermal injection of Tomaralimab effectively improved AD-like skin inflammation in BALB/c mice exposed to house dust mites (11). However, it remains unclear whether allergenic hapten-induced AD can be effectively treated with a systemically administered humanized monoclonal Ab targeting TLR2. The primary objective of the present study was to evaluate the therapeutic potential of systemically administered Tomaralimab for treating AD-like skin inflammation triggered by the contact allergen 2,4-dinitrochlorobenzene (DNCB) in BALB/c mice.

Materials and methods

Materials. Tomaralimab (OPN-305; cat. no. NM-103), a humanized anti-TLR2 Ab, was provided by Neuramedy Co., Ltd. DNCB (cat. no. 237329), toluidine blue (TB; cat. no. 89640) and hematoxylin (cat. no. H3136) and eosin B (cat. no. 212954) were purchased from MilliporeSigma. Anti-thymic stromal lymphopoietin (TSLP) Ab (cat. no. NBP1-76754) was obtained from Novus Biologicals, LLC and anti-HMGB1 Ab (cat.

Correspondence to: Professor Soon Young Shin, Department of Biological Sciences, Sanghuh College of Life Science, Konkuk University, 120 Neungdong, Gwangjin, Seoul 05029, Republic of Korea
E-mail: shinsy@konkuk.ac.kr

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no. ab18256) was obtained from Abcam. Anti-IL1β Ab (cat. no. 2022) and anti-F4/80 Ab (cat. no. 70076) were obtained from Cell Signaling Technology, Inc., and anti-IL33 Ab (cat. no. 12372-1-AP) was purchased from Proteintech Group, Inc. Anti-MPO Ab (code no. A0398) was obtained from Dako; Agilent Technologies, Inc. Anti-GAPDH (cat. no. sc-32233) and anti-IL17 (cat. no. sc-374218) Abs were purchased from Santa Cruz Biotechnology, Inc. Anti-IL31 Ab (cat. no. 701082) and secdondary Abs conjugated with horseradish peroxidase (anti-rabbit IgG, cat. no. 31460; anti-mouse IgG, cat. no. 31430) and rhodamine Red-X (cat. no. R6394) were obtained from Thermo Fisher Scientific, Inc.

Induction of AD-like skin lesions in a mouse model. A total of 18 male BALB/c mice (body weight range, 18-23 g), aged ~7 weeks, were purchased from Orient Bio, Inc. The mice were housed in a controlled environment at 22±2˚C with constant humidity (40-60%), maintained under a 12-h light-dark cycle. They were provided with unrestricted access to sterile water and food in a specific pathogen-free grade laboratory. In the induction of AD with DNCB in BALB/c mice, 4% SDS and 1% DNCB are typically used initially to create skin barrier disruption and sensitization, respectively (12,13). The commonly used dose of neutralizing Abs for intravenous (IV) administration in mice usually ranges from 1 to 25 mg/kg (14,15). In preliminary experiments, two doses of Tomaralimab were tested, 25 and 50 mg/kg. It was observed that administrating 25 mg/kg effectively reduced DNCB-induced skin inflammation, comparable to a higher dose of 50 mg/kg. Consequently, the 25 mg/kg dosage of Tomaralimab was selected for investigation. The mice were divided into three groups in a random manner: Group I, Control; Group II, DNCB + PBS; and Group III, DNCB + Tomaralimab (n=6 in each group). The method of inducing skin inflammation similar to AD through the topical application of DNCB was carried out as previously described (16). Group I mice were treated with a vehicle (acetone:olive oil, 3:1 v/v), and Group II and III mice were sensitized with 4% SDS on the dorsal skin to disrupt the skin barrier. After 4 h, SDS-sensitized areas were subjected to daily topical application of 1% DNCB in vehicle for 3 days. Following a 4-day break, 0.5% DNCB was applied topically to the same area seven times at 2-day intervals, and the mice were sacrificed the next day (on day 20). Group III mice received an IV dose of Tomaralimab (25 mg/kg) following each DNCB application. On day 20, all mice were euthanized in a chamber with CO_2 at a fill rate of 50% of the chamber volume per min. After visual confirmation of death, the mice were further exposed to CO_2 for an additional minute to ensure the absence of a heartbeat for confirmation of complete euthanasia and final death. The animal studies followed the guidelines approved (IACUC; approval number KU26036) by the Konkuk University Institutional Animal Care and Use Committee (Seoul, Korea).

Tissue preparation and histopathologic examination. Skin lesions from the dorsal surface were surgically excised and fixed overnight at 25˚C with 100% acetone, then embedded in paraffin. Paraffin blocks were sliced at a thickness of 5 μm with a microtome (Leica Microsystems GmbH). As previously described, paraffin-embedded tissues were deparaffinized with xylene and rehydrated using a graded series of ethanol (17). Rehydrated sections were immersed in hematoxylin solution for 1 min at 25˚C, rinsed under running tap water, and subsequently immersed in eosin solution for 30 sec at 25˚C. Dehydration was performed by passing the sections through a graded ethanol series (80, 90, 95 and 100%) for 30 sec each at 25˚C. The slides were then rinsed in xylene three times for 5 min each at 25˚C and mounted with coverslips.

The mast cells infiltrated were stained with 0.1% TB for 3 min at 25˚C. Stained images were observed using a light microscope (EVOS FL Auto; Thermo Fisher Scientific, Inc.). Epidermal thickness was measured using ImageJ 1.52a software (National Institutes of Health).

Immunohistochemical staining. After blocking the intracellular peroxidase activity for 1 h at 25˚C with 3% hydrogen peroxide, tissue sections were incubated overnight at 4˚C with primary antibodies against F4/80 (1:200) and MPO (1:500), followed by avidin/biotin complex-mediated DAB staining using VECTASTAIN Elite ABC-HRP Kit (cat. no. PK-6101; Vector Laboratories, Inc.), and counterstained with hematoxylin, as previously described (11).

Fluorescent immunohistochemical staining was carried out as previously described (11). A secondary Ab labeled with rhodamine Red-X (1:500) was incubated for 1 h at 25˚C for the fluorescence staining. Hoechst 33258 (10 μg/ml) was employed to counterstain the nuclear DNA. Subsequently, the slides were treated with a fluorescence mounting medium (ProLong Gold Antifade Reagent; Invitrogen; Thermo Fisher Scientific, Inc.) after washing with PBS. Fluorescence images were taken with an EVOS FL Auto system, and the fluorescence levels were quantified using ImageJ software (National Institutes of Health).

Stimulation of HaCaT cells. Human keratinocyte HaCaT cells were obtained from the CLS Cell Line Service GmbH. The cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (HyClone; Cytiva) and penicillin-streptomycin (Sigma-Aldrich; Merck KGaA) in a humidified atmosphere with 5% CO_2 at 37˚C. DNCB were treated with varying concentrations (0-50 nM) for 18 h (for detecting mRNA levels) and 24 h (for detecting protein levels), or 10 nM DNCB for various periods (0-36 h). Tomaralimab at concentrations of 0.5, 1, 5 and 10 μg/ml was pretreated 30 min before DNCB stimulation.

Immunoblotting. HaCaT cells were lysed in lysis buffer (50 mM Tris-HCl pH 7.4, 400 mM NaCl, 1% NP-40, 0.25% sodium deoxycholate, 1 mM EDTA, 1 mM NaF, 1 mM $NaVO_4$) as previously described (18). The proteins (20 μg/lane) were electrophoresed on 10% SDS-PAGE and transferred to nitrocellulose membranes. After incubation with the specific primary Abs overnight at 4˚C, HRP-conjugated secondary Abs (goat anti-rabbit IgG, 1:8,000; goat anti-mouse IgG, 1:8,000) were incubated for 1 h at 25˚C. The blots were visualized utilizing an enhanced chemiluminescence (ECL) detection system (cat. no. 34580; Thermo Fisher Scientific, Inc.). Densitometric analysis was performed using ImageJ 1.52a software (National Institutes of Health).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA isolation, cDNA synthesis, and PCR

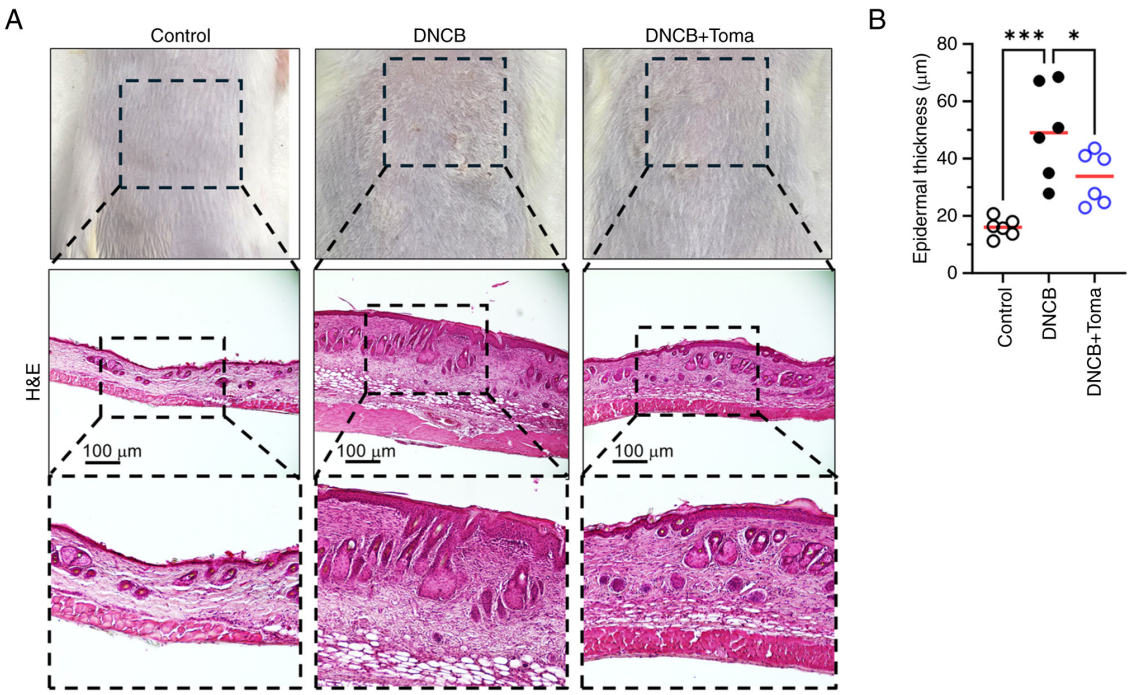

Figure 1. Effect of systemic administration of Toma on DNCB-induced skin lesions in BALB/c mice. (A) Paraffin-embedded skin tissues of BALB/c mice were prepared on day 20; H&E staining was performed. (B) Epidermal thickness was measured in digital images using ImageJ 1.52a software. Scale bars, 100 μm. Data are expressed as the mean ± standard deviation (n=6). *P<0.05 and ***P<0.001 by Dunnett's multiple comparisons test. Toma; Tomaralimab; DNCB, 2,4-dinitrochlorobenzene; H&E, hematoxylin and eosin.

reaction were carried out as previously described (19). The PCR primers used in the present study were as follows: TSLP forward, 5'-TAGCAATCGGCCACATTGCCT-3' and reverse, 5'-GAAGCGACGCCACAATCCTTG-3'; IL1β forward, 5'-AAACAGATGAAGTGCTCCTTCCAGG-3' and reverse, 5'-TGGAGAACACCACTTGTTGCTCCA-3'; IL17 forward, 5'-CCATAGTGAAGGCAGGAA TC-3' and reverse, 5'-GAG GTGGATCGGTTGTAGTA-3'; IL31 forward, 5'-TCGAGG AATTACAGTCCCTCT-3' and reverse, 5'-TGTCGAGGTGCT CTATGATCTC-3'; IL33 forward, 5'-CAAAGAAGTTTG CCCCATGT-3' and reverse, 5'-AAGGCAAAGCACTCCACA GT-3'; HMGB1 forward, 5'-ATATGGCAAAAGCGGACA AG-3' and reverse, 5'-AGGCCAGGATGTTCTCCTTT-3'; and GAPDH forward, 5'-ACCCACTCCTCCACCTTTG-3' and reverse, 5'-CTCTTGTGCTCTTGCTGGG-3'. The amplified PCR products were visualized under UV transillumination.

Cell counting kit-8 (CCK-8) assay. Cell viability was measured using a EZ-Cytox assay kit (cat. no. EZ-3000; DoGenBio). HaCaT cells cultured in 96-well plates (5,000 cells/100 μl) were treated with varying concentrations of DNCB (0, 5, 10, 25 and 50 nM) for 24 h. The assay solution (10 μl per 100 μl culture medium) was added to each plate and incubate for 1 h in a 37˚C CO_2 incubator, after which the absorbance was measured at 450 nm using an Emax Endpoint ELISA Microplate Reader (Molecular Devices, LLC).

Statistical analysis. The data are presented as the average value ± standard deviation (SD). To compare multiple groups, a one-way ANOVA followed by Dunnett's multiple comparisons test or Tukey's multiple comparisons test was performed using GraphPad Prism V10.1.2 software (GraphPad Software Inc.; Dotmatics). P<0.05 was considered to indicate a statistically significant difference.

Results

IV administration of Tomaralimab attenuates DNCB-induced AD-like skin lesions in BALB/c mice. To assess the therapeutic effectiveness of systemic administration of Tomaralimab for allergenic hapten-induced AD, a mouse model was used, in which the allergenic hapten DNCB was applied topically to the back skin of BALB/c mice (11). Topical application of DNCB leads to typical AD-like skin inflammation with superficial redness and edema (11,20). Under these experimental conditions, Tomaralimab was injected IV through the lateral tail vein every other day until day 19 (Fig. S1). H&E staining revealed that DNCB significantly increased epidermal hyperplasia, which was reduced by systemic administration of Tomaralimab (Fig. 1A). Measurement of epidermal thickness using ImageJ demonstrated that Tomaralimab administration resulted in a significant reduction (P=0.043 by Dunnett's multiple comparisons test, n=6) in DNCB-induced epidermal thickness (Fig. 1B).

IV administration of Tomaralimab restores filaggrin (FLG) and TSLP expression in DNCB-induced AD-like skin lesions in BALB/c mice. FLG is an epidermal protein responsible for maintaining skin barrier function (21), and its deficiency contributes critically to AD (22). TSLP is an epithelial-derived pro-inflammatory cytokine that triggers dendritic cells, T-lymphocytes and mast cells to stimulate the release of various inflammatory cytokines and is considered a crucial marker in the early pathogenesis of AD (23,24). Immunofluorescence

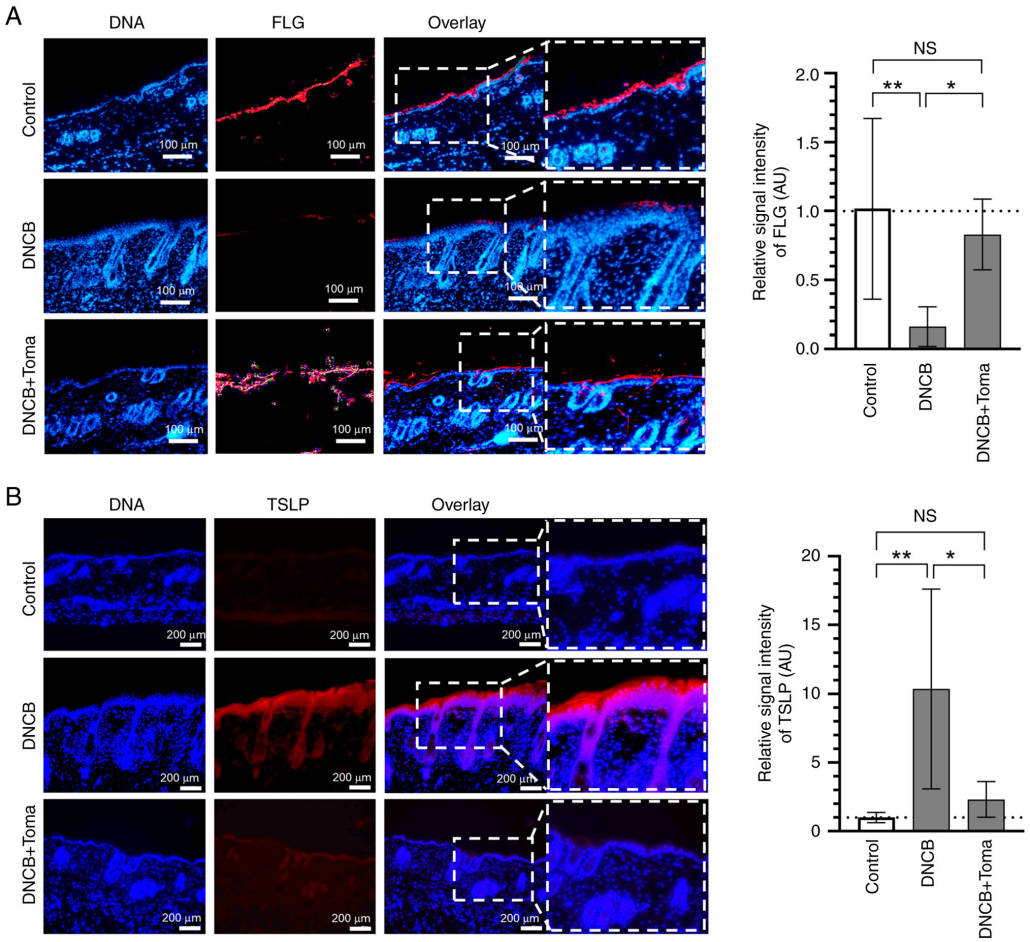

Figure 2. Effect of systemic administration of Toma on DNCB-induced FLG and TSLP expression in BALB/c mice. (A and B) Paraffin-embedded tissue sections prepared on day 20 were subjected to immunofluorescence staining with (A) anti-FLG and (B) anti-TSLP antibodies with a rhodamine-red-X-conjugated secondary antibody (red). The nuclei were counterstained with Hoechst 33258 (blue). Scale bars, 100 μm. ImageJ software was used to measure fluorescent signals (right graphs). The data are expressed as the mean ± SD. (n=6). *P<0.05 and **P<0.01 by Tukey's multiple comparisons test. Toma; Tomaralimab; DNCB, 2,4-dinitrochlorobenzene; FLG, filaggrin; TSLP, thymic stromal lymphopoietin; NS, not significant.

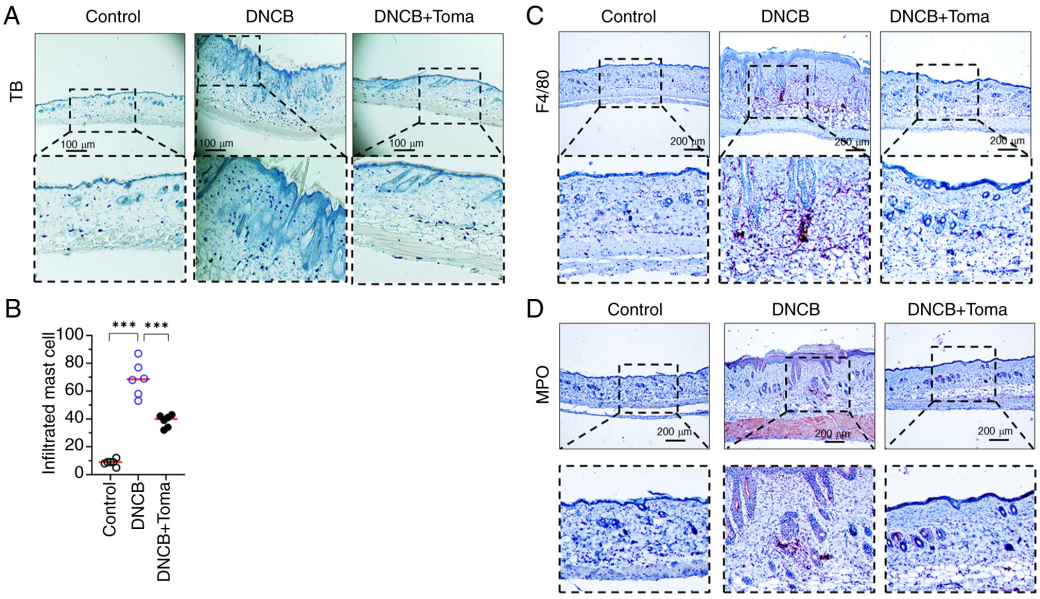

Figure 3. Effect of systemic administration of Toma on DNCB-induced infiltration of immune cells in BALB/c mice. (A) Paraffin-embedded tissue sections prepared on day 20 were stained with TB. The blue spots indicate the infiltrated mast cells. Scale bars, 100 μm. (B) The number of TB-positive mast cells was counted. The data are expressed as the mean ± SD (n=6). ***P<0.001 by Dunnett's multiple comparisons test. (C and D) Immunohistochemical staining of paraffin-embedded tissue sections prepared on day 20 for (C) F4/80-positive macrophages and (D) MPO-positive basophils/neutrophils. Scale bars, 200 μm. Toma, Tomaralimab; DNCB, 2,4-dinitrochlorobenzene; TB, toluidine blue; MPO, myeloperoxidase.

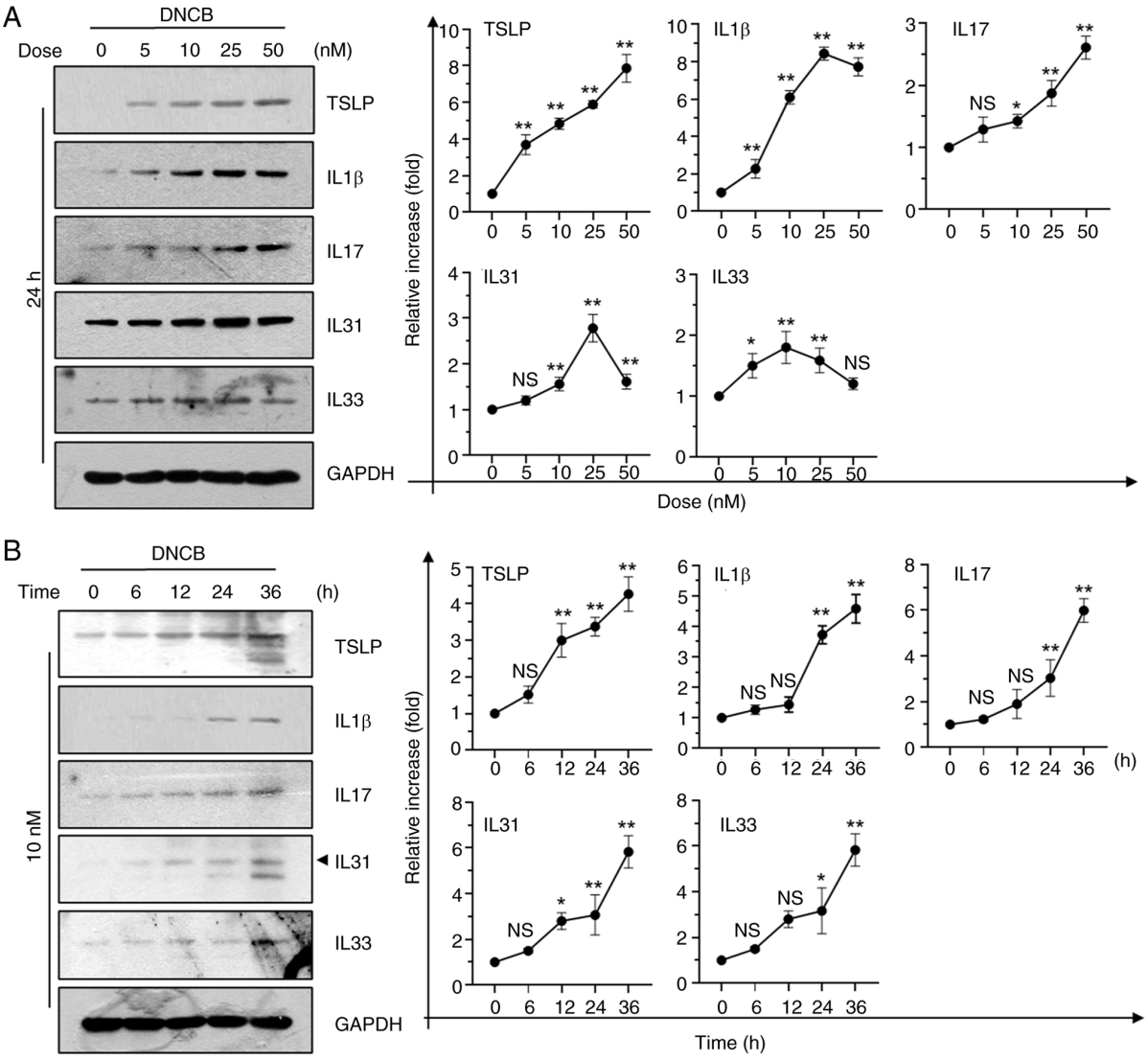

Figure 4. Effect of DNCB on the expression of pro-inflammatory cytokines in HaCaT keratinocytes. (A and B) The effect of DNCB on the expression of pro-inflammatory cytokines was examined using immunoblot analysis based on different (A) doses and (B) time-points. The band intensities were measured using ImageJ software. The data are expressed as the mean ± SD (n=3). *P<0.05 and **P<0.01 by Dunnett's multiple comparisons test. DNCB, 2,4-dinitrochlorobenzene; TSLP, thymic stromal lymphopoietin; IL, interleukin; NS, not significant.

staining revealed that administration of Tomaralimab led to the recovery of DNCB-induced suppression of FLG levels (Fig. 2A) and decreased DNCB-induced TSLP expression (Fig. 2B). These data suggest that systemic administration of Tomaralimab has a potential impact on the recovery of damaged skin barrier function and inhibiting the onset of allergic skin inflammation.

IV administration of Tomaralimab decreases the infiltration of inflammatory cells in DNCB-induced AD-like skin lesions in BALB/c mice. The increased infiltration of immune cells, including T lymphocytes and mast cells, at inflammatory sites is closely linked to the pathogenesis of AD (25). It was also observed that the administration of Tomaralimab significantly (P<0.001 by Dunnett's multiple comparisons test, n=6) reduced the population of infiltrated mast cells stained by TB (Fig. 3A and B). Furthermore, Tomaralimab led to a notable decrease in the presence of F4/80-positive macrophages (Fig. 3C) and myeloperoxidase (MPO)-positive immune cells, including basophils and neutrophils (Fig. 3D).

Tomaralimab decreases the expression of DNCB-induced pro-inflammatory cytokines in HaCaT keratinocytes. Keratinocytes play crucial roles in the pathogenesis of AD (26). To investigate how Tomaralimab impacts DNCB-induced inflammation on a cellular level,inflammatory responses in HaCaT keratinocytes were triggered using DNCB. Previous studies have demonstrated that keratinocytes release multiple pro-inflammatory cytokines involved in the pathogenesis of AD, including TSLP, IL-1β, IL-17 and IL-33 (16,27). Immunoblot analysis demonstrated that DNCB increased levels of TSLP, IL-1β, IL-17, IL-31 and IL-33 in a dose- (Fig. 4A) and time-dependent manner (Fig. 4B). When HaCaT cells were exposed to varying concentrations (0-50 nM) of DNCB, no cytotoxicity was observed; instead, DNCB exposure exhibited slight increases in survival rate (Fig. S2). Certain cytokines, such as IL-1β and IL-31, reached peak levels when the DNCB concentration exceeded 25 nM, and all tested cytokines approached sub-peak levels after 24 h of treatment. Cells were treated at a concentration of 10 nM for 24 h to

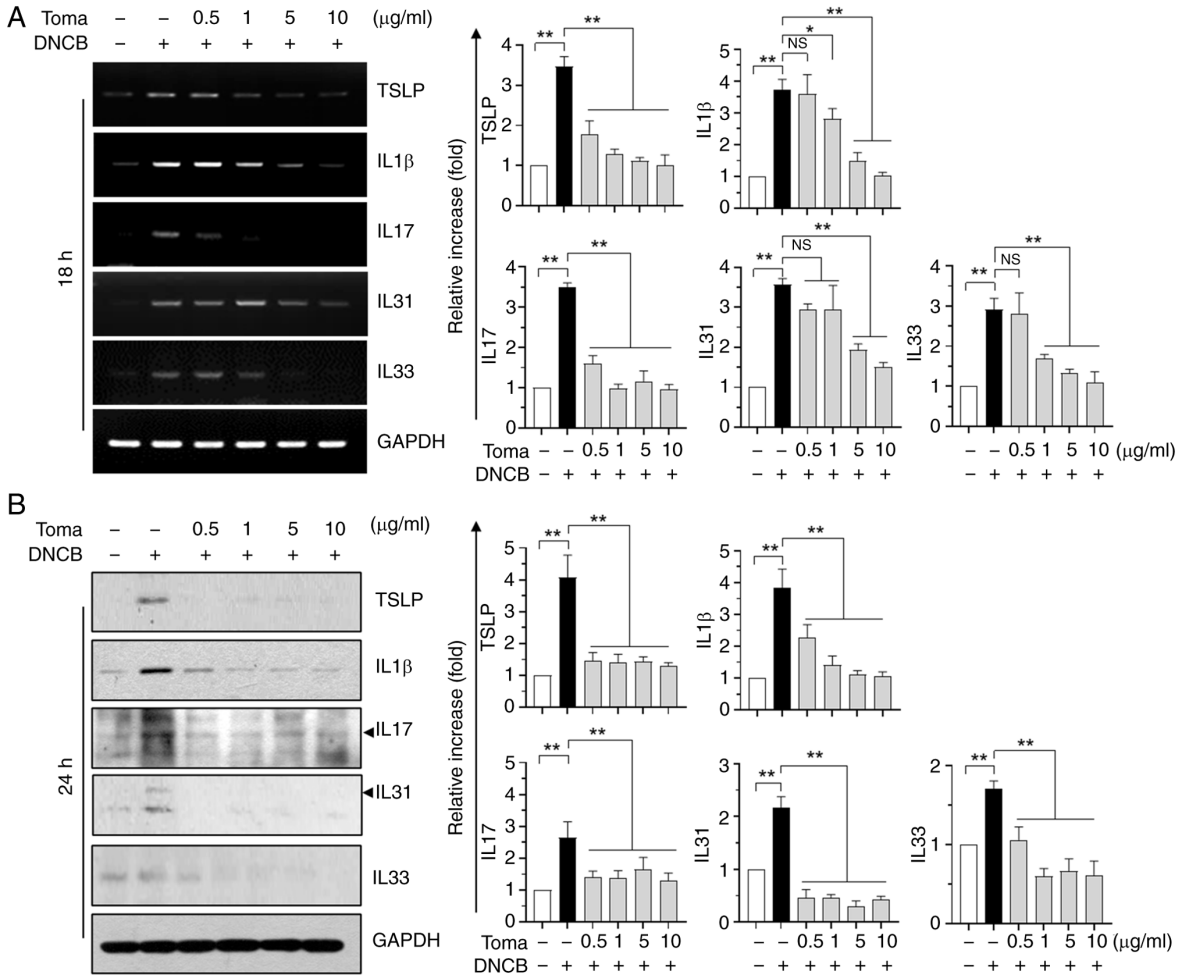

Figure 5. Effect of Toma on the inhibition of DNCB-induced pro-inflammatory cytokines in HaCaT keratinocytes. (A and B) The effect of Tomaralimab on the inhibition of DNCB-induced pro-inflammatory cytokines was examined using (A) reverse transcription-quantitative PCR and (B) immunoblot analysis. The band intensities were measured using ImageJ software. The data are expressed as the mean ± SD (n=3). *P<0.05 and **P<0.01 by Dunnett's multiple comparisons test. Toma, Tomaralimab; DNCB, 2,4-dinitrochlorobenzene; TSLP, thymic stromal lymphopoietin; IL, interleukin; NS, not significant.

maximize the inhibitory action of Tomaralimab. Under these experimental conditions, Tomaralimab treatment resulted in a dose-dependent decrease in mRNA levels of DNCB-induced inflammatory cytokines (Fig. 5A). In line with the RT-qPCR findings, the protein levels of inflammatory cytokines were reduced in a dose-dependent manner (Fig. 5B).

Tomaralimab inhibits DNCB-induced high mobility group box 1 (HMGB1) expression in HaCaT keratinocytes. HMGB1 is a chromatin-binding nuclear protein involved in DNA bending and assembly of proteins at specific DNA sites (28). HMGB1 can be produced from necrotic cells and implicated as a mediator of inflammation by induction of various pro-inflammatory cytokines (29). AS HMGB1 is an endogenous TLR2 ligand involved in TLR2-induced inflammation (30), it was examined whether DNCB induces HMGB1 expression. It was observed that DNCB increased mRNA (Fig. 6A) and protein (Fig. 6B) levels in a time-dependent manner. Under this experimental condition, treatment with Tomaralimab dose-dependently reduced DNCB-induced HMGB1 expression at mRNA (Fig. 6C) and protein (Fig. 6D) levels. To evaluate the impact of Tomaralimab on HMGB1 expression *in vivo*, immunofluorescence analysis was conducted on skin tissues from BALB/c mice challenged with DNCB. Topical application of DNCB markedly increased the staining intensity of HMGB1 in the epidermis (Fig. 6E, left panel). By contrast, the administration of Tomaralimab led to a significant decrease in DNCB-induced HMGB1 intensity (P<0.05, n=6) (Fig. 6E, right graph). These data suggest that the decrease in HMGB1 levels may be associated with the inhibition of DNCB-induced skin lesions by the humanized anti-TLR2 Ab, Tomaralimab.

Discussion

At least 10 TLRs (TLR1-10) have been identified (31). A strong connection has been observed between the quantity of TLR2 ligands in the skin and the extent of severity of AD (32). Also, innate TLR2 ligands promote Th2 cell-mediated chronic AD (33). These findings emphasize the pivotal role of TLR2 in the development of AD and show promise as a possible target for AD treatment. Previously, it was demonstrated that intradermal injection of Tomaralimab effectively relieved AD-like skin inflammation in BALB/c mice exposed to house dust mite extracts (11), offering promise for AD treatment through systemic Tomaralimab therapy. However, it remains unclear whether allergenic hapten-induced AD can be effectively

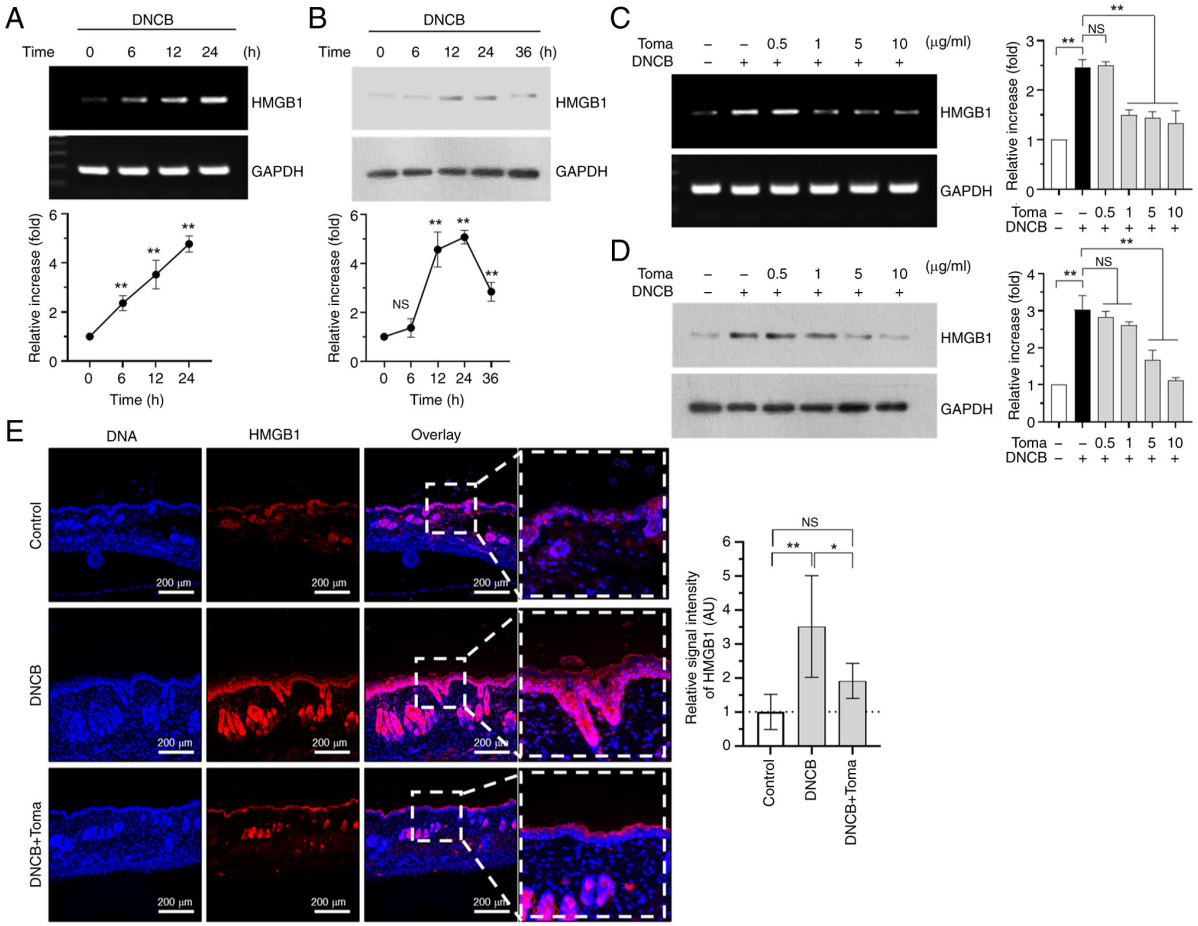

Figure 6. Effect of Toma on the inhibition of DNCB-induced HMGB1 expression in HaCaT keratinocytes. (A and B) The effect of DNCB on the expression of HMGB1 was examined using (A) RT-qPCR and (B) immunoblot analysis. (C-E) The effect of Toma on the inhibition of DNCB-induced HMGB1 expression was examined using (C) RT-qPCR, (D) immunoblot analysis and (E) fluorescent immunohistochemistry. The band and fluorescent intensities were measured using ImageJ software. The data are expressed as the mean ± SD (n=3 for A-D, n=6 for E). *P<0.05 and **P<0.01 by Dunnett's multiple comparisons test (A-D) or Tukey's multiple comparisons test (E). Toma, Tomaralimab; DNCB, 2,4-dinitrochlorobenzene; HMGB1, High mobility group box 1; RT-qPCR, reverse transcription-quantitative PCR; NS, not significant.

treated with a systemically administered humanized monoclonal Ab targeting TLR2. In the present study, it was aimed to elucidate the effects of systemic Tomaralimab administration on DNCB-induced AD in a BALB/c mouse model.

The skin lesions in AD are characterized by marked infiltration of inflammatory cells, including mast cells and T-lymphocytes, and the concurrent production of large amounts of various inflammatory cytokines (34). In the induction of AD with DNCB in BALB/c mice, 4% SDS and 1% DNCB were used to create skin barrier disruption and sensitization (12,13). SDS is a surfactant that disrupts the skin barrier, facilitating the penetration of DNCB into the skin. This disruption helps ensure that the DNCB adequately triggers the immunological response. The higher concentration of DNCB (>1%) in the initial applications sensitizes the immune system effectively. This first exposure is critical as it primes the immune system by creating an allergic response, stimulating T-cells, and setting the stage for an inflammatory reaction typical of AD (12). After sensitization, a lower concentration of DNCB (0.5%) is used in repeated applications to sustain the inflammatory response. This lower concentration can trigger an immune response in already-sensitized skin without causing excessive irritation or toxicity, allowing for a controlled and sustained AD-like condition in the mice. Therefore, the combination of 4% SDS and higher concentrations of DNCB initially followed by a lower concentration helps establish a robust model of AD that mimics both the sensitization and chronic phases of the disease (12). The findings of the present study demonstrated that systemic administration of Tomaralimab in BALB/c mice effectively alleviated DNCB-induced AD-like skin lesions by reducing the infiltration of inflammatory cells, including TB-positive mast cells, F4/80-positive macrophages and MPO-positive neutrophils and basophils, into skin lesions and suppressing the production of multiple inflammatory cytokines closely associated with the pathogenesis of AD. Furthermore, administration of Tomaralimab reduces DNCB-induced TSLP expression, crucial for mast cell development, immune responses mediated by mast cells, initiating Th2 response, and upregulating itch-related factors such as IL-31 and IL-33, responsible for activating sensory neurons to trigger itchiness (35-38). These results demonstrate that the administration of Tomaralimab effectively reduces the pathogenesis of hapten-induced AD in a BALB/c mouse model.

The mechanism by which TLR2-targeting Abs suppress hapten-induced skin inflammation remains largely unknown. Continual contact with chemical allergens on the skin may

lead to the secretion of TLR ligands, such as DAMP molecules, from injured cells (39). HMGB1, a molecule known as a DAMP molecule, binds to various TLRs, including TLR2 (40), and functions as a pro-inflammatory mediator to initiate inflammation by promoting the production of multiple inflammatory cytokines (41). In this context, it was demonstrated that the mRNA expression level of *HMGB1* was enhanced by DNCB and dose-dependently reduced by Tomaralimab, suggesting that TLR2-targeting Abs could block the inflammatory response elicited by allergenic hapten-induced DAMPs. The current results support the notion that TLR2 plays a crucial role in allergenic hapten-induced AD-like skin inflammation and that targeting TLR2 with humanized monoclonal Abs is a feasible therapeutic approach. Further studies are necessary to explore the effects of targeting TLR2 on skin inflammation induced by multiple allergenic haptens in the pathogenesis of AD.

In conclusion, the present findings further support the feasibility of Ab therapy using humanized anti-TLR2 monoclonal Ab, Tomaralimab, as a promising candidate for systemic therapy in treating allergenic hapten-induced AD-like skin disorders.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

HY carried out biochemical analysis, animal experiments, histological examination, ethodology, formal analysis and data curation. EJ conducted investigation, histological examination and data curation. TYK performed formal analysis and visualization SYS conceptualized and supervised the study, acquired funding, wrote, reviewed and edited the manuscript. All authors read and approved the final version of the manuscript. All athors confirm the authenticity of all the raw data.

Ethics approval and consent to participate

All animal studies were conducted following the guidelines for animal experiments and procedures approved (IACUC; approval no. KU26036) by the Konkuk University Institutional Animal Care and Use Committee (Seoul, Korea).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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