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Inhibition of Sphingosine-1-Phosphate Receptor 2 ($S1P_2$) Attenuates Imiquimod-Induced Psoriasis-Like Skin Inflammation in BALB/c Mice

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Abstract

Serum and epidermal levels of sphingosine 1-phosphate (S1P) are higher in patients with psoriasis than healthy subjects. Although roles of type 1 S1P receptor, $S1P_1$, in the development of psoriasis has intensively been investigated, roles of $S1P_2$ have not been elucidated. We aim to investigate whether blockage of $S1P_2$ reduce imiquimod-induced psoriasis-like dermatitis using an $S1P_2$ antagonist, JTE-013, in combination with *S1pr2* wild-type (WT) and knock-out (KO) BALB/c mice. Imiquimod induced increase of erythematous papules and plaques with silver scaling, whereas administration of JTE-013 significantly suppressed those increases in *S1pr2* WT mice. Deficiency of *S1pr2* gene reduced the imiquimod-induced symptoms. Imiquimod increased mRNA expression levels of pro-inflammatory Th1/Th17 cytokines, whereas JTE-013 significantly suppressed those increases in *S1pr2* WT mice. Deficiency of *S1pr2* gene also suppressed the imiquimod-induced pro-inflammatory cytokine expression. Imiquimod induced enlargement of lymph nodes and spleens, whereas JTE-013 suppressed them in *S1pr2* WT mice. Imiquimod induced increase of pro-inflammatory Th1/Th17 cytokine levels and Th17 cell numbers in lymph nodes and spleens, whereas JTE-013 suppressed them in *S1pr2* WT mice. In summary, the present results suggest that blockage of $S1P_2$ could suppress the characteristics of psoriasis-form dermatitis and be a therapeutic strategy.

Key Words: Sphingosine 1-phosphate, $S1P_2$, Psoriasis, Dermatitis, JTE-013, Immune suppression

INTRODUCTION

Psoriasis is a chronic inflammatory and proliferative skin disease caused by environmental and genetic factors and is characterized by excessive infiltration of immune cells in the dermis and epidermis (Masuda-Kuroki *et al*., 2023; Zhao *et al*., 2023). Abnormal immune responses play important roles in the pathogenesis of psoriasis, especially the IL-23/IL-17 axis, which is a key driver in the development of psoriatic inflammation (Zhao *et al*., 2023). Several studies have reported higher levels of circulating sphingosine 1-phosphate (S1P) in patients with psoriasis than healthy subjects (Checa *et al*., 2015; Myśliwiec *et al*., 2017; Kozłowska *et al*., 2019). Levels of sphingosine and S1P were higher in psoriatic epidermis than non-affected epidermis (Moon *et al*., 2013; Matwiejuk *et al*., 2023). S1P is an intercellular lipid mediator that regulates various pathophysiological processes through specific G protein-coupled receptors, $S1P_{1\text{-}5}$ (Park and Im, 2017).

Type 1 S1P receptor, $S1P_1$ modulators and sphingosine kinase 2 inhibitors alleviated psoriasis-like dermatitis in mice (Liu *et al*., 2021). The mechanism of $S1P_1$ modulators in treating psoriasis might be related to a decrease in the number of white blood cells, percentage of $CD3^+$ T cells, and IL-23 mRNA levels (Liu *et al*., 2021). For examples, fingolimod (FTY720), the first approved oral immunosuppressant targeting $S1P_{1/3/4/5}$, exerted immunosuppressive effects by suppressing lymphocyte efflux (di Nuzzo *et al*., 2014), and ameliorated imiquimod-induced psoriasis-form dermatitis histologically and clinically (Okura *et al*., 2021). In a psoriasis model, expanded γδT17 cells egress from lymph nodes and migrate to inflamed skin in a $S1P_1$-depdendent manner (Ramírez-Valle *et al*., 2015). Oral administration of Syl930, a selective $S1P_1$ modulator, induced strong anti-inflammatory and anti-proliferative effects in multiple psoriasis models (Ji *et al*., 2018). IMMH002, another $S1P_1$ modulator, significantly ameliorated psoriasis PASI scores and pathological damage (Jin *et al*., 2020). Topical in-

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hibition of S1P production improved inflammation in psoriasis-like mice, possibly by blocking Th17 cell differentiation (Shin *et al*., 2019). In a phase II trial, ponesimod, a $S1P_1$ modulator, showed therapeutic potential for psoriasis (Vaclavkova *et al*., 2014). Topical application of HWG-35D, an inhibitor of sphingosine kinase 2, improved imiquimod-induced psoriatic lesions in mice by reducing IL-17A levels (Shin *et al*., 2020). However, functions of $S1P_2$ have not been investigated in psoriasis models, although blockage of $S1P_2$ attenuated mast cell aggregation and reduced inflammatory cytokine levels in murine allergic asthma and atopic dermatitis models (Park and Im, 2019, 2020; Kang *et al*., 2020). Therefore, in the present study, we aim to investigate whether inhibition of $S1P_2$ could reduce imiquimod-induced psoriasis-like dermatitis using an $S1P_2$ antagonist, JTE-013, in combination with *S1pr2* wild-type (WT) and knock-out (KO) BALB/c mice.

MATERIALS AND METHODS

JTE-013 was purchased from Cayman Chemicals (Ann Arbor, MI, USA). Other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Animals

Three *S1pr2* gene heterozygous mice were kindly provided by Richard Proia at NIH (Kono *et al*., 2007). They had been backcrossed to BALB/c mice from Orient (Seoul, Korea) for eight generations. *S1pr2* wild-type littermates (WT) and knockout (KO) mice were housed in the Laboratory Animal facility at Kyung Hee University and provided with food and water *ad libitum*. Eight-week-old male *S1pr2* WT and KO BALB/c mice were used. The animal protocol used in this study was reviewed by the Institutional Animal Care Committee of Kyung Hee University and approved with respect to the ethics of procedures and care of animals (Approval Number, KH-SASP-24-196).

Induction of psoriasis in mice and JTE-013 administration

Following a simple randomization procedure, 8-week-old male BALB/c *S1pr2* WT and KO mice were randomly assigned to one of the following treatment groups (n=5): a vaseline-treated control group, an imiquimod-treated group, and a JTE-013 plus imiquimod-treated group. On day 0, the back skin of mice was shaved, and commencing on day one, 62.5 mg of 5% imiquimod cream (Aldara cream, Dong-A ST) was applied to the exposed skin daily for 6 days to induce psoriasis-like skin inflammation (Son *et al*., 2022). JTE-013 (3 mg/kg) was administered via an intraperitoneal injection 30 min prior to the application of the imiquimod cream. The mice were sacrificed on day 7 (Lee *et al*., 2024).

Measurement of PASI

Inflammation of the back skin of mice was scored daily during the experimental period based on the Psoriasis Area and Severity Index (PASI). The PASI scoring system includes assessments of erythema, scaling, and thickening, each of which is rated on a scale from 0 to 4 based on severity (0, none; 1, slight; 2, moderate; 3, marked; 4, very marked). The total score was obtained by accumulating the scores obtained for each of the three indices, thereby giving a total score of between 0 and12 (Son *et al*., 2022).

Histological assessment of psoriasis

Specimens obtained from the back skin of mice were fixed in 10% formalin and dehydrated in a 30% sucrose solution for 24 h at 4°C. The tissue samples were then embedded in O.C.T. compound. Section (8 μm) were stained with H&E. For immunohistochemical staining of the proliferation marker Ki-67, the sections were washed with PBS containing 0.5% Tween-20 (PBS-T) and blocked with PBS-T containing 5% bovine serum albumin for 30 min at room temperature. After blocking, the sections were labeled with Ki-67 recombinant rabbit monoclonal antibody (MA5-14520, Invitrogen, Carlsbad, CA, USA) for 1 h at room temperature, and thereafter stained using a VECTASTAIN® Elite ABC Kit (PK-6101, Vector Laboratories, Burlingame, CA, USA). The peroxidase reaction product was visualized by incubating slides with ImmPACT® DAB Substrate (SK-4105, Vector Laboratories) for 10 min at room temperature, followed by counterstaining with hematoxylin (Lee and Im, 2024).

Quantitative real-time PCR

To assess the expression of inflammatory markers in the skin, lymph nodes, and spleens, we performed RT-PCR. First-strand cDNA was synthesized from total RNA isolated using a TRIzol reagent (Invitrogen, Waltham, MA, USA); Total RNA was isolated from the lymph nodes, spleens, and skin tissues and mRNA was transcribed to cDNA with Moloney Murine Leukemia Virus Reverse Transcriptase (Promega, Madison, WI, USA). Quantitative PCR was performed using Thunderbird Next SYBR qPCR Mix (Toyobo, Osaka, Japan) in conjunction with a CFX Connect Real-Time System (Bio-Rad, Hercules, CA, USA). The thermal-cycling conditions were as follows: an initial cycle at 95°C for 4 min, followed by 40 cycles at 95°C for 30 s and at 57°C for 30 s, and a final single cycle at 95°C for 30 s. Analysis of the data was performed using the $2^{-\Delta\Delta Ct}$ method with CFX Maestro Software (Bio-Rad). The expression of individual genes was normalized against levels of the Gapdh reference gene (Kang *et al*., 2024).

Flowcytometric analysis

To determine T cell population, single cells isolated from lymph nodes or spleens were stained with an FITC-labeled rat antibody against CD4 (cat. 11-0041-82, eBioscience, San Diego, CA, USA) at 4°C for 20 min. The cells were fixed at room temperature for 1 h using an intracellular fixation buffer (cat. 00-8222-49, eBioscience). After fixation, the cells were permeabilized using a permeabilization buffer (cat. 88-8824- 00, eBioscience) and stained at room temperature for 1 h with APC-labeled rat anti-Foxp3 (cat. 17-5773-82, eBioscience), with eFluor 450-labeled rat anti-T-bet (cat. 48-5825-82, eBioscience) or with eFluor 660-labeled rat anti-IL-17A (cat. 50-7177-82, eBioscience). The cells were analyzed using a CytoFLEX Flow cytometer (Beckman Coulter, Brea, CA, USA) (Park *et al*., 2024).

T cell differentiation

Naïve $CD4^+$ T cells were isolated from mouse splenocytes using magnetic beads (Naïve $CD4^+$ T Cell Isolation Kit, Miltenyi Biotec, Bergisch Gladbach, Germany). These cells were then placed in Th2 cell differentiation media in 24-well plates that had been coated with anti-mouse CD3 and CD28 antibodies and cultured for three days. The Th2 differentiation medium included recombinant human IL-2 (Peprotech 200-02),

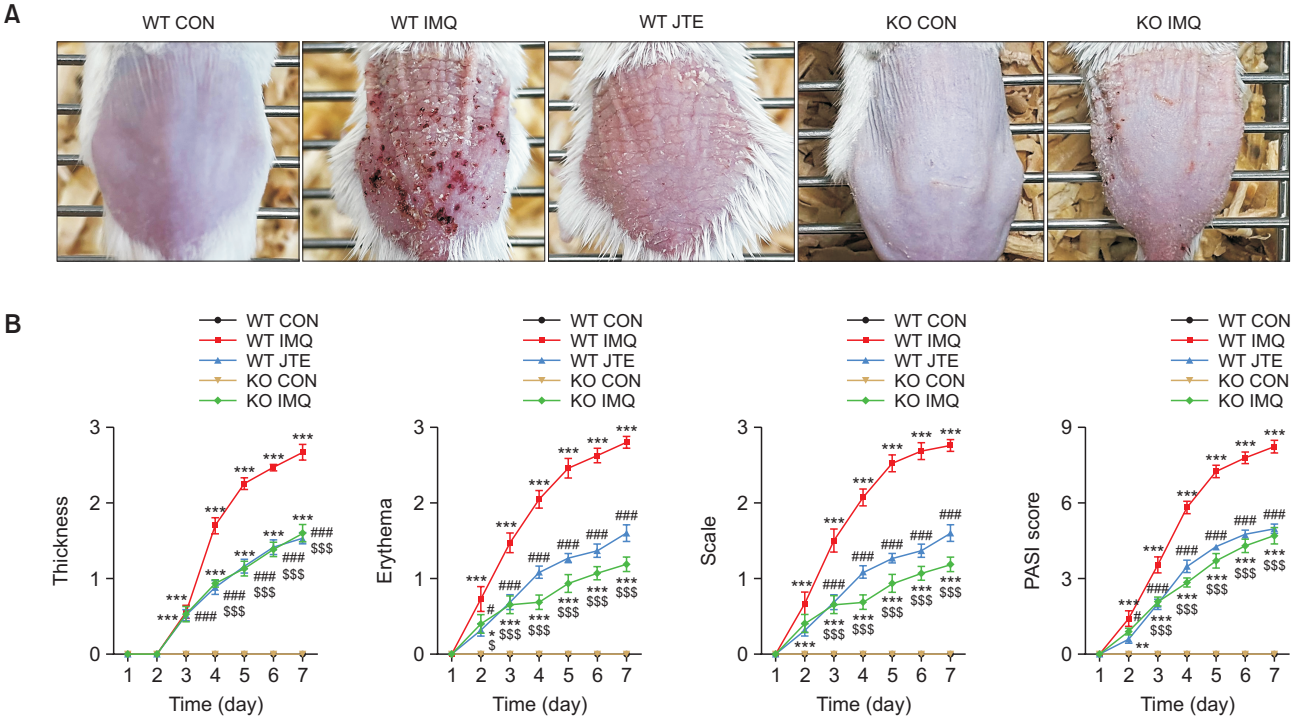

Fig. 1. Administration of JTE-013 suppressed imiquimod-induced psoriasis-like skin inflammation in *S1pr2* WT mice. A murine model of imiquimod-induced psoriasis was established through consecutive imiquimod application for 6 days; JTE-013 (3 mg/kg, i.p.) was administrated 30 min before imiquimod application to *S1pr2* WT BALB/c mice or in *S1pr2* KO mice (A and B). Macroscopic views of mouse back skin after 6 days of treatment (A). Thickening, erythema, scale, and PASI score, which is the sum of thickening, erythema, and scaling scores on the seventh day (B). The results are presented as the means ± SEM (n=5). ***p<0.001 vs. the vehicle-treated group, $^{\#\#\#}p$<0.001 vs. the imiquimod-treated group, $^{\$\$\$}p$<0.001 vs. the imiquimod-treated groups of *S1pr2* WT mice.

recombinant mouse IL-4 (Peprotech 214-14), and anti-mouse IFN-γ (BioXcell BE0055). On the third day, fresh Th cell differentiation media was added, and the cells were cultured for an additional three days. On day six, the cells were collected 4-6 hours after treatment with Golgi inhibitors and restimulation with anti-CD3, and Th cell differentiation was assessed using flow cytometry. JTE-013 at concentrations of 10 and 30 μM was added to the Th2 differentiation media to determine its effect (Lee and Lee, 2024).

Verification of normality and statistical analysis

The results obtained from animal experiments are expressed as means ± standard error of the mean (SEM) of eight measurements. To assess the normality of data distribution, we performed a Kolmogorov–Smirnov (KS). The statistical significance of differences was determined using an analysis of variance (ANOVA) and Tukey's multiple comparison test. Statistical significance was set at p-value <0.05. Normality assessment and statistical analyses were performed using GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

Administration of JTE-013 or *S1pr2* gene deficiency suppresses imiquimod-induced psoriasis in BALB/c mice

We investigated the effects of JTE-013 on the pathogenesis of psoriasis by treating *S1pr2* WT BALB/c mice with JTE-013 in an *in vivo* imiquimod-induced psoriasis model. After 6 days of treatment, imiquimod-induced psoriatic skin lesions, scale-covered erythematous plaques, were clearly observed, whereas JTE-013 administration suppressed the erythematous papules and plaques with silver scaling in *S1pr2* WT mice (Fig. 1A). Three days after the start of imiquimod application on the shaved back skin, signs of thickening, erythema, and scaling appeared, which continually increased in severity until the end of the experiment in *S1pr2* WT mice (Fig. 1B). However, in the mice administered JTE-013, there was a significant reduction in these pathological features and, consequently, the associated PASI scores in *S1pr2* WT mice (Fig. 1A, 1B). In *S1pr2* KO BALB/c mice, after 6 days of treatment, imiquimod-induced psoriatic skin lesions were less clearly observed compared to *S1pr2* WT mice (Fig. 1A). That is, *S1pr2 gene deficiency* resulted in suppression of the erythematous papules and plaques with silver scaling, observed as significant reduction of signs and the PASI scores in *S1pr2* KO mice (Fig. 1B).

Administration of JTE-013 or *S1pr2* gene deficiency suppresses imiquimod-induced keratinocyte proliferation in BALB/c mice

In response to sustained skin inflammation, inflammatory cytokines (TNF-α, IL-17, and IFN-γ) can promote the uncontrolled proliferation of keratinocytes thereby leading to the development of acanthosis (epidermal hyperplasia) (Rendon

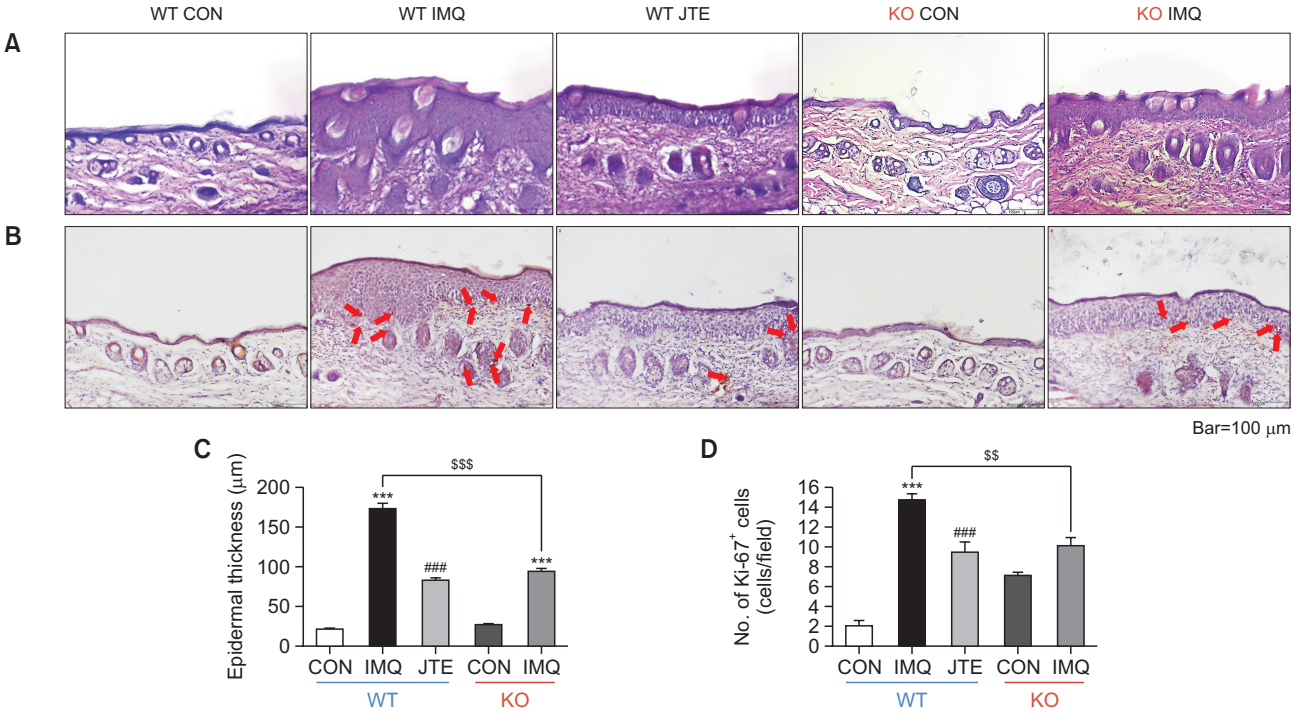

Fig. 2. Administration of JTE-013 suppressed imiquimod-induced pathologic skin changes in *S1pr2* WT mice. Imiquimod was applied on the dorsal skin consecutively for 6 days; JTE-013 (3 mg/kg, i.p.) was administrated 30 mins before imiquimod application to *S1pr2* WT mice or in *S1pr2* KO mice (A-D). (A) H&E staining of the back skin. In the imiquimod group, we detected lengthening and clubbing of the rete ridges and moderate-to-severe dermal lymphocyte infiltrates. (B) Ki-67 staining. (C) Epidermal thickness. (D) Histograms of Ki-67$^+$ cell numbers. ***p<0.001 vs. the vehicle-treated group, $^{###}p$<0.001 vs. the imiquimod-treated group, $^{\$\$\$}p$<0.001, $^{\$\$}p$<0.01 vs. the imiquimod-treated groups of *S1pr2* WT mice.

and Schäkel, 2019). Skin samples were stained with H&E and Ki-67 to perform histological analyses of skin tissues. Compared with the control group mice, H&E staining revealed a thickening of the epidermis due to hyperkeratosis in the imiquimod-treated mice compared to the control group (Fig. 2A). The extensive hypertrophy of the epidermis induced by imiquimod was significantly suppressed following JTE-013 treatment in *S1pr2* WT BALB/c mice (Fig. 2A), the results of which are presented quantitatively as histograms of epidermal tissue thickness in Fig. 2C. The major symptom of psoriasis is keratinocyte hyperproliferation. Compared with the control group, the imiquimod group showed a significant increase in Ki-67-positive keratinocytes (Fig. 2B), whereas the administration of JTE-013 proved to be effective in reducing this increase in Ki-67-positive cells in *S1pr2* WT mice (Fig. 2B, 2D). On the other hand, in *S1pr2* KO mice, imiquimod-induced increase in Ki-67-positive keratinocytes and epidermal thickness were significantly suppressed in *S1pr2* KO mice compared to the imiquimod-treated group in *S1pr2* WT mice (Fig. 2B, 2D).

Administration of JTE-013 or *S1pr2* gene deficiency suppresses imiquimod-induced changes in Th1 and Th17 cytokines in the skin of BALB/c mice

Given that psoriasis is considered to be regulated by Th1 and Th17 responses, we also sought to aseess the expression levels of inflammatory Th1 cytokine (*Il-1β*, *Tnf-α*, *Il-6*, and *Ifn-γ*) and Th17 cytokine (*Il-22* and *Il-17a*) mRNAs in the skin of imiquimod-treated BALB/c mice. We accordingly detected significantly elevated levels of the mRNAs of all the assessed cytokines in skin tissues, whereas JTE-013 administration significantly suppressed these increases in *S1pr2* WT mice (Fig. 3A-3F). We also examined the expression levels of filaggrin (*FLG2*), which plays roles in skin barrier function and is a constituent of the keratinocyte membrane, and thymic-stromal-lymphopoietin (*TSLP*), which is expressed in keratinocytes and activates the maturation of epidermal Langerhans cells. Whereas treatment with imiquimod was found to induce increases in the mRNA levels of *FLG2* and *TSLP*, the administration of JTE-013 was observed to significantly suppress these increases in *S1pr2* WT mice (Fig. 3G, 3H). In *S1pr2* KO mice, the mRNA levels of Th1 and Th17 cytokines were not significantly increased in the skin after imiquimod treatment (Fig. 3A-3F). In addition, the mRNA levels of *FLG2* and *TSLP* were also not increased in *S1pr2* KO mice (Fig. 3G, 3H). These results thus provided evidence to indicate that imiquimod-induced psoriasis is associated with elevated levels of Th1 and Th17 cytokines and barrier function proteins, and the administration of JTE-013 or deficiency of *S1pr2* is effective in suppressing these changes, thereby indicating its anti-psoriasis efficacy.

Administration of JTE-013 or *S1pr2* gene deficiency suppresses imiquimod-induced increases in weights of lymph nodes and spleen in BALB/C mice

Next, we checked the sizes of the lymph nodes as well as spleens. Draining lymph nodes orchestrates an immune response in the skin. Lymph nodes become swollen when an infection or an immune reaction occurs. In the imiquimod-in-

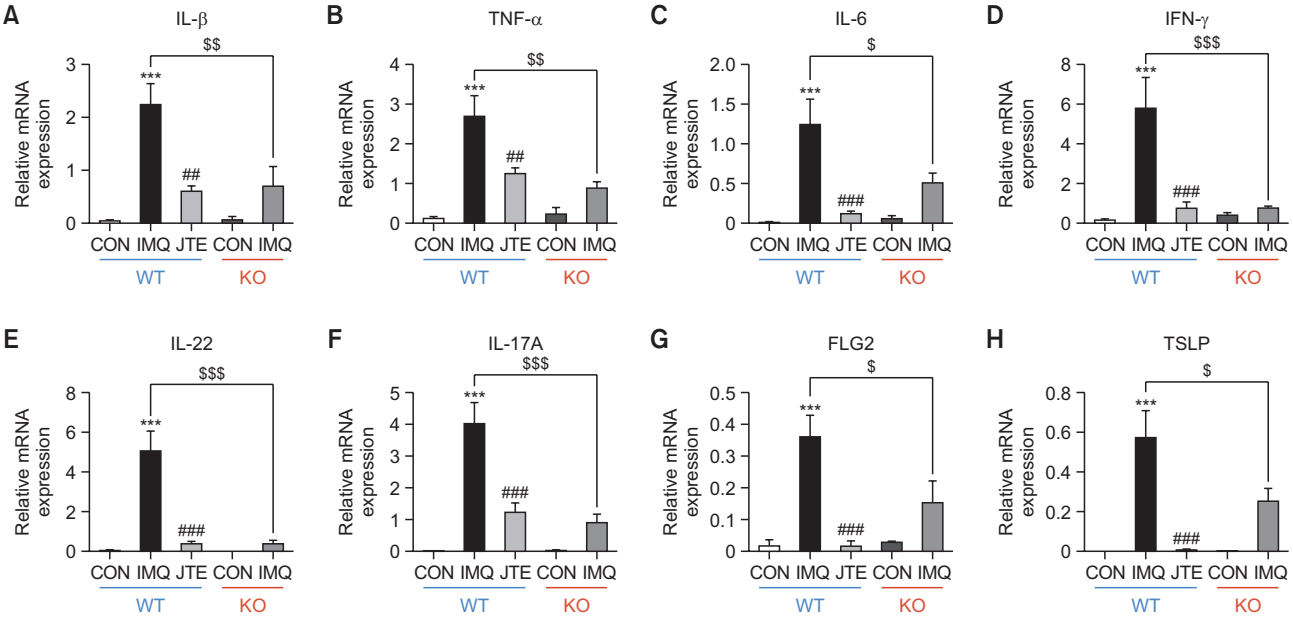

Fig. 3. Effect of JTE-013 on the expression levels of the Th1 and Th17 cytokines in the skin tissues of imiquimod-induced psoriasis in *S1pr2* WT and KO mice. qRT-PCR analysis of the mRNA expression of Th1 (IL-1β, TNF-α, IL-6, and IFN-γ) and Th17 (IL-22 and IL-17A) cytokines, *FLG2*, and *TSLP* in skin tissues from the imiquimod-induced and JTE-013-treated *S1pr2* WT mice or in *S1pr2* KO mice. (A) IL-1β, (B) TNF-α, (C) IL-6, (D) IFN-γ, (E) IL-22, (F) IL-17A, (G) *FLG2*, and (H) *TSLP*. The relative mRNA levels of the cytokines were quantified by determining the ratios of their levels to GAPDH transcript levels. The results are presented as the mean ± SEM (n=5). ***p<0.001 vs. the vehicle-treated group, $^{###}p$<0.001, $^{##}p$<0.01 vs. the imiquimod-treated group, $^{\$\$\$}p$<0.001, $^{\$\$}p$<0.01, $^{\$}p$<0.05 vs. the imiquimod-treated groups of *S1pr2* WT mice.

duced psoriasis mouse group, the lymph nodes were much more swollen than those in the control group, as manifested by increased lymph node weights (Fig. 4A, 4C). JTE-013 significantly suppressed the imiquimod-induced increase in lymph node weight in *S1pr2* WT mice (Fig. 4A, 4C). In *S1pr2* KO mice, the lymph nodes were swollen compared to those in the control group, however, the increment was less than that in *S1pr2* WT mice (Fig. 4A, 4C). The spleen is analogous to the lymph nodes and plays an important role in the immune response. Splenomegaly can result from infections or immune reactions. In the imiquimod-induced psoriasis mouse group, the spleens become enlarged along with increased weights compared to those in the control group (Fig. 4B, 4D), whereas the administration of JTE-013 was found to significantly suppress these changes in spleens in *S1pr2* WT mice (Fig. 4B, 4D). In *S1pr2* KO mice, the spleens were swollen compared to those in the control group, however, the increment was less than that in *S1pr2* WT mice (Fig. 4B, 4D).

Administration of JTE-013 or *S1pr2* gene deficiency suppresses imiquimod-induced increases in Th1 and Th17 cytokine expression in lymph nodes and spleens

We detected elevated mRNA expression levels of the inflammatory Th1 (*Il-1β*, *Tnf-α*, *Il-6*, and *Ifn-γ*) and Th17 (*Il-22* and *Il-17a*) cytokines in the lymph nodes after psoriasis induction (Fig. 5), whereas JTE-013 treatment suppressed these increases in *S1pr2* WT mice (Fig. 5). In *S1pr2* KO mice, the mRNA expression levels of Th1 and Th17 cytokines were not increased after imiquimod treatment (Fig. 5).

The levels of inflammatory Th17 cytokines (*Il-22* and *Il-17a*) and Th1 cytokines (*Il-1β*, *Tnf-α*, *Il-6*, and *Ifn-γ*) were also measured in the spleens. The mRNA levels of these cytokines were significantly elevated in the lymph nodes after psoriasis induction in *S1pr2* WT mice, whereas JTE-013 treatment suppressed the elevation of cytokine levels (Fig. 6). In *S1pr2* KO mice, the levels of inflammatory Th17 and Th1 cytokines in the spleens were not increased after psoriasis induction (Fig. 6).

Administration of JTE-013 or *S1pr2* gene deficiency suppresses imiquimod-induced increases in populations of Th17 cells in lymph nodes and spleens

The numbers of $CD4^+$T-bet^+ Th1 cells, $CD4^+$IL-$17A^+$ Th17 cells, and $CD4^+FoxP3^+$ Treg cells in the lymph nodes was also measured. According to FACS analysis of isolated cells from the draining lymph nodes, the populations of Th1, Th17, and Treg cells increased by imiquimod, where JTE-013 suppressed only the increase in Th17 cell populations in *S1pr2* WT mice (Fig. 7A-7C). In *S1pr2* KO mice, the populations of Th1 cells increased by imiquimod, but the populations of Th17 and Treg cells were not significantly changed (Fig. 7A-7C).

The number of Th1 cells, Th17 cells, and Treg cells in the spleen was measured. According to FACS analysis of isolated cells from the spleen, the populations of Th1 and Th17 cells increased by imiquimod but not Treg cells (Fig. 7D-7F), whereas JTE-013 suppressed the increase in Th1 and Th17 cell populations in *S1pr2* WT mice (Fig. 7D, 7E). In *S1pr2* KO mice, the populations of Th1 cells increased significantly by imiquimod, but the populations of Th17 and Treg cells were not significantly changed (Fig. 7A-7C).

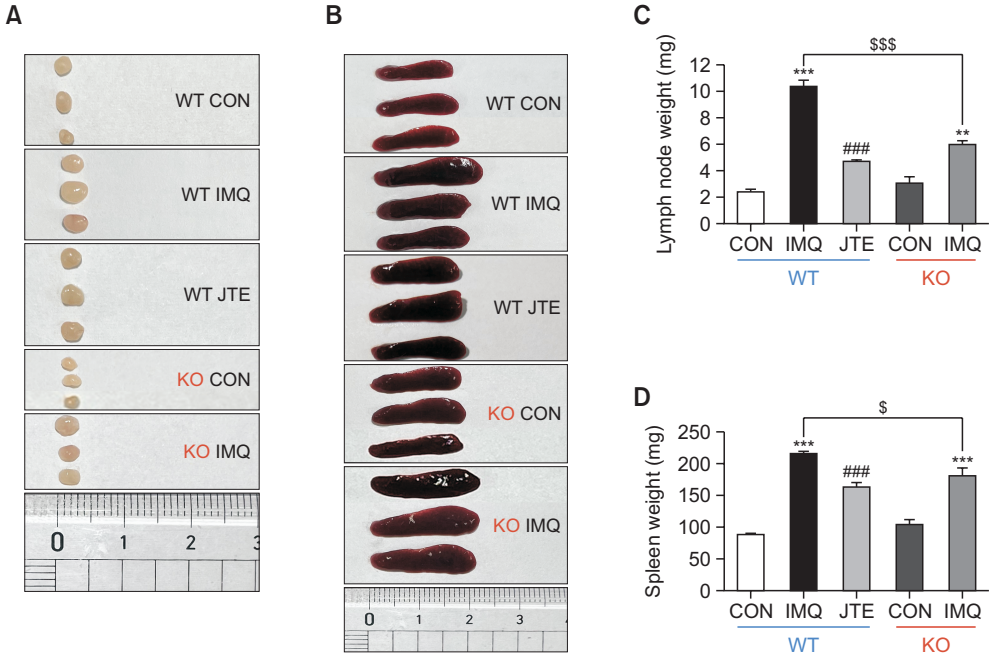

Fig. 4. Effect of JTE-013 on the lymph node sizes and spleens sizes in *S1pr2* WT and KO mice. (A) Images of the draining lymph nodes. (B) Images of the spleens. (C) The measured weights of lymph nodes. (D) The measured weights of spleens. The results are presented as the mean ± SEM (n=5). ***p<0.001, **p<0.01 vs. the vehicle-treated group, $^{###}p$<0.001 vs. the imiquimod-treated group, $^{\$\$\$}p$<0.001, $^{\$}p$<0.05 vs. the imiquimod-treated groups of *S1pr2* WT mice.

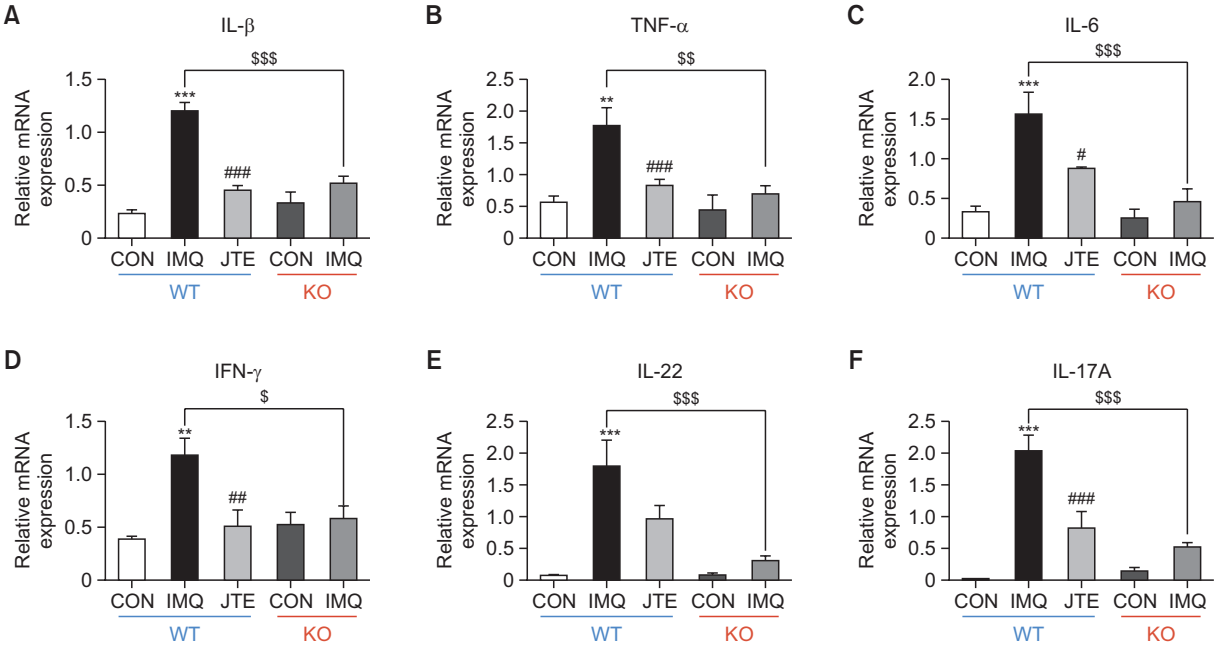

Fig. 5. Effect of JTE-013 on the expression levels of the Th1 and Th17 cytokines in the lymph nodes of imiquimod-induced psoriasis in *S1pr2* WT and KO mice. qRT-PCR analysis of the mRNA expression of Th1 (IL-1β, TNF-α, IL-6, and IFN-γ) and Th17 (IL-22 and IL-17A) cytokines in lymph nodes from the imiquimod-induced and JTE-013-treated *S1pr2* WT or KO groups. (A) IL-1β, (B) TNF-α, (C) IL-6, (D) IFN-γ, (E) IL-22, (F) IL-17A. The relative mRNA levels of the cytokines were quantified by determining the ratios of their levels to GAPDH transcript levels. The results are presented as the mean ± SEM (n=5). ***p<0.001, **p<0.01 vs. the vehicle-treated group, $^{###}p$<0.001, $^{##}p$<0.01, $^{#}p$<0.05 vs. the imiquimod-treated group, $^{\$\$\$}p$<0.001, $^{\$\$}p$<0.01, $^{\$}p$<0.05 vs. the imiquimod-treated groups of *S1pr2* WT mice.

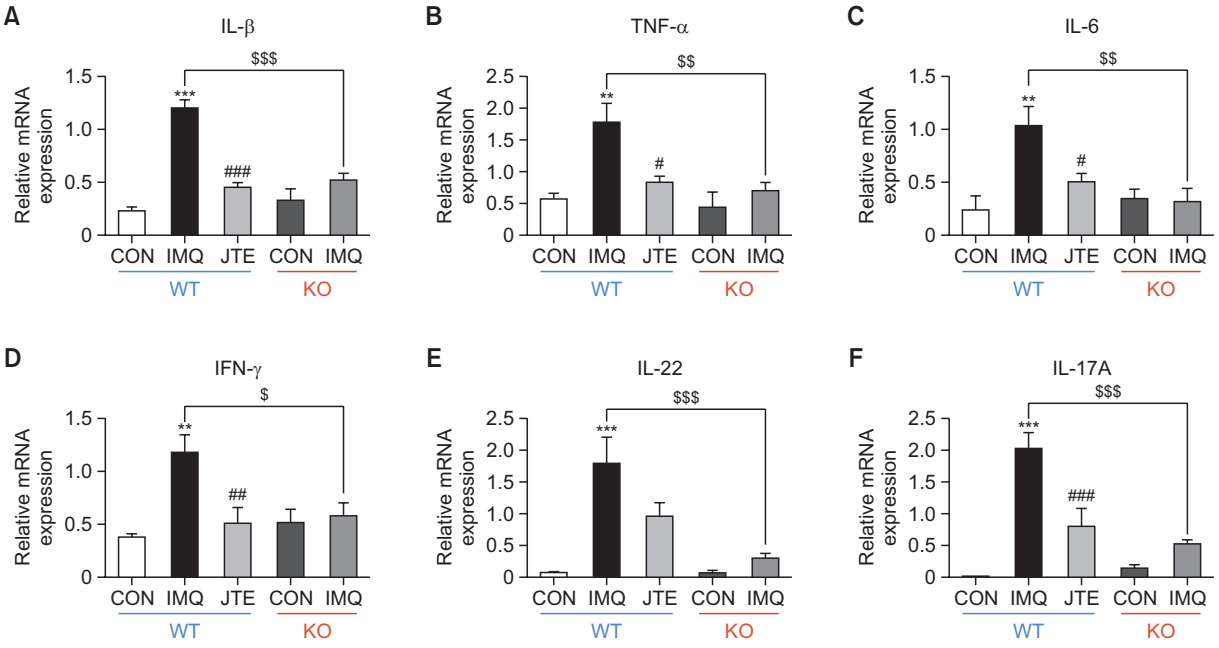

Fig. 6. Effect of JTE-013 on the expression levels of the Th1 and Th17 cytokines in the spleens of imiquimod-induced psoriasis in *S1pr2* WT and KO mice. qRT-PCR analysis of the mRNA expression of Th1 (IL-1β, TNF-α, IL-6, and IFN-γ) and Th17 (IL-22 and IL-17A) cytokines in lymph nodes from the imiquimod-induced and JTE-013-treated *S1pr2* WT or KO groups. (A) IL-1β, (B) TNF-α, (C) IL-6, (D) IFN-γ, (E) IL-22, (F) IL-17A. The relative mRNA levels of the cytokines were quantified by determining the ratios of their levels to GAPDH transcript levels. The results are presented as the mean ± SEM (n=5). ***p<0.001, **p<0.01 vs. the vehicle-treated group, $^{###}p$<0.001, $^{##}p$<0.01, $^{#}p$<0.05 vs. the imiquimod-treated group, $^{\$\$\$}p$<0.001, $^{\$\$}p$<0.01, $^{\$}p$<0.05 vs. the imiquimod-treated groups of *S1pr2* WT mice.

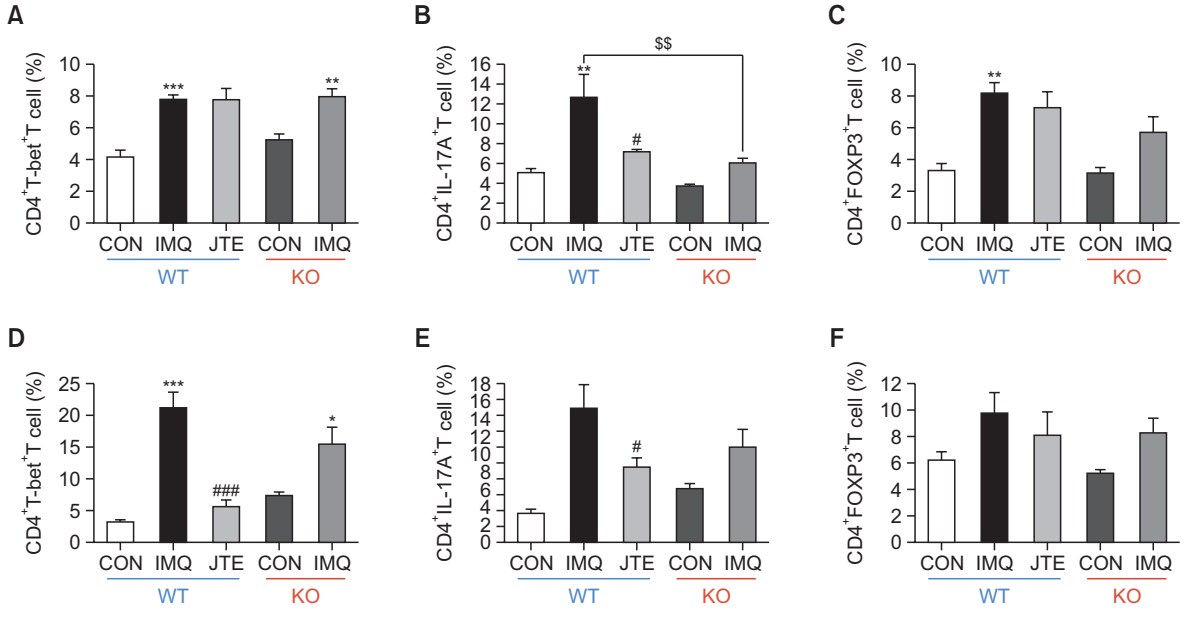

Fig. 7. Effect of JTE-013 on T cell populations in *S1pr2* WT and KO mice. (A) Percentage of $CD4^+T\text{-}bet^+$ Th1 cells in the lymph nodes. (B) Percentage of $CD4^+IL\text{-}17+$ Th17 cells in the lymph nodes. (C) Percentage of $CD4^+FoxP3^+$ Treg cells in the lymph nodes. (D) Percentage of $CD4^+T\text{-}bet^+$ T cells in the spleens. (E) Percentage of $CD4^+IL\text{-}17+$ Th17 cells in the spleens. (F) Percentage of $CD4^+FoxP3^+$ Treg cells in spleens. The results are presented as the mean ± SEM (n=5). ***p<0.001, **p<0.01, *p<0.05 vs. the vehicle-treated group, $^{###}p$<0.001, $^{#}p$<0.05 vs. the imiquimod-treated group, $^{\$\$}p$<0.01 vs. the imiquimod-treated groups of *S1pr2* WT mice.

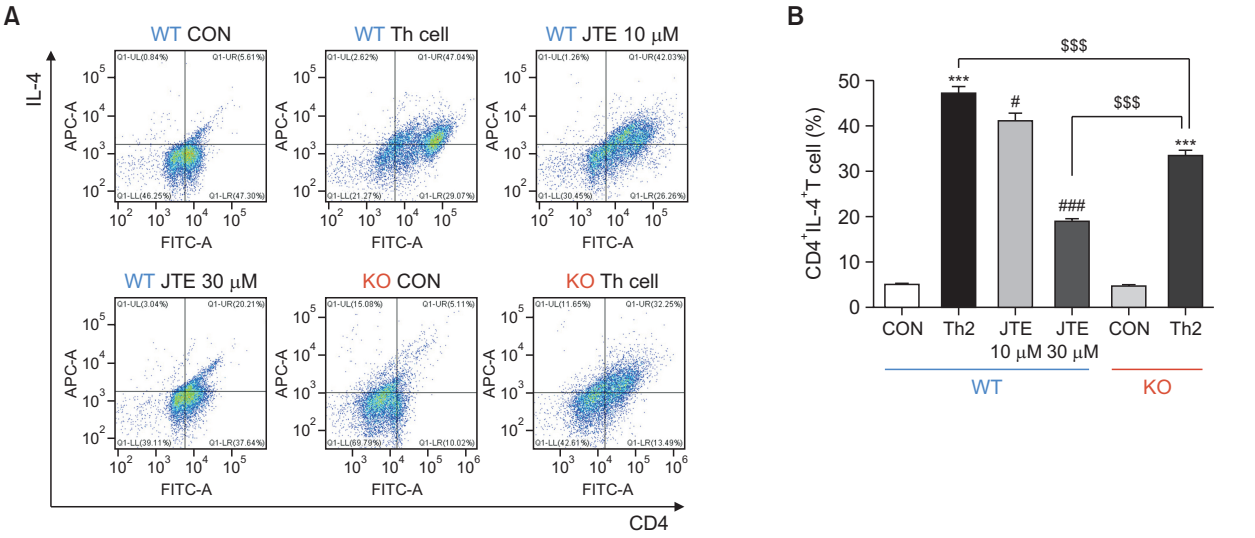

Fig. 8. Suppressive effect of JTE-013 on T cell differentiation into Th2 cells. $CD4^+$ T cells, isolated from splenocytes, were cultured in media for Th2 cell differentiation for 5 days in plates pre-coated with an antibody to mouse CD3. Representative flowcytometry results for $CD4^+IL\text{-}4^+$ Th1 cell differentiation (A) Histograms show the percentage of $CD4^+IL\text{-}4^+$ cells (B) (n=5). ***p<0.001 vs. the vehicle-treated group, ###p<0.001, #p<0.05 vs. the imiquimod-treated group, $^{\$\$\$}p$<0.001, $^{\$\$}p$<0.01 vs. the imiquimod-treated groups of *S1pr2* WT mice.

Treatment of JTE-013 or *S1pr2* gene deficiency suppresses T cells differentiation into Th2 cells

In a recent study on collagen-induced rheumatoid arthritis, administration of JTE-013 or *S1pr2* gene deficiency reduced differentiation of naïve T cells into $IL\text{-}17A^+$ Th17 cells and $IFN\text{-}\gamma^+$ Th1 cells in a concentration-dependent manner *in vitro* (Lee *et al*., 2024). Therefore, we investigated whether JTE-013 treatment or *S1pr2* gene deficiency could suppress the T cell differentiation into Th2 cells. As shown in Fig. 8, JTE-013 treatment of Th2 differentiation media significantly suppressed the differentiation into $IL\text{-}4^+$ Th2 cells in a concentration-dependent manner *in vitro* (Fig. 8A, 8B). Furthermore, we assessed the effects of *S1pr2* gene deficiency on the differentiation into $IL\text{-}4^+$ Th2 cells and found that deficiency reduced $IL\text{-}4^+$ Th2 cell generation compared to naïve T cells from WT mice (Fig. 8A, 8B). In summary, administration of JTE-013 or *S1pr2* gene deficiency reduces the differentiation into $IL\text{-}4^+$ Th2 cells in addition to differentiation into $IFN\text{-}\gamma^+$ Th1 and $IL\text{-}17A^+$ Th17 cells (Lee *et al*., 2024).

DISCUSSION

In the present study, for the first time, the pro-inflammatory functions of $S1P_2$ in psoriasis-like dermatitis were elucidated using JTE-013 treatment and *S1pr2*-deficient mice. Three main $S1P_2$ functions were found in imiquimod-induced psoriasis-like dermatitis. First, $S1P_2$ exacerbated inflammatory psoriasis-like dermatitis *in vivo* by up-regulating Th1 and Th17 cytokines in the skin. Second, blockade of $S1P_2$ could be used as a potential therapeutic strategy for the treatment of psoriasis-like dermatitis, which was proven with JTE-013 treatment. Third, $S1P_2$-mediated pro-inflammatory immune activation might be resulted from increases in Th1 and Th17 cytokines, and Th17 cell numbers in lymph nodes and spleens.

Keratinocytes, the most important cells in the psoriasis, have been reported to express five S1P receptors, $S1P_{1\text{-}5}$ (Igawa *et al*., 2019). Previously, Kim *et al*. reported that S1P inhibited keratinocyte proliferation by inhibiting the Akt/PKB pathway (Kim *et al*., 2004). Jeon *et al*. demonstrated that elevated S1P concentration by downregulating S1P degrading enzyme led to keratinocyte differentiation (Jeon *et al*., 2020). S1P and its signaling inhibited the proliferation of keratinocytes and induced their differentiation (Masuda-Kuroki and Di Nardo, 2022). $S1P_2$ was found to be dominantly involved in the S1P-induced keratinocyte growth arrest by dephosphorylation of Akt (Schüppel *et al*., 2008). Thus, S1P and $S1P_2$ have been implicated in keratinocytes differentiation and growth arrest. However, in the imiquimod-induced psoriasis-like model, keratinocytes have to proliferate and thicken epidermis. This might be resulted from signals of pro-inflammatory Th1 and Th17 cytokines induced by imiquimod treatment.

Actually, S1P stimulated the production of inflammatory cytokines, such as TNF-α, IL-8, and IL-36γ in normal human epidermal keratinocytes via $S1P_1$ and $S1P_2$ (Igawa *et al*., 2019). Treatment with *Staphylococcus aureus* bacterial supernatant increased the transcription of $S1P_2$ in keratinocytes and JTE-013 blocked the secretion of inflammatory cytokines (Igawa *et al*., 2019). Blocking S1P significantly reduced swelling, inflammatory cell infiltration, and edema in the ear skin for psoriasis-like skin lesions (Schaper *et al*., 2013). In the present study, JTE-013 treatment or deficiency of *S1pr2* suppressed the expression levels of pro-inflammatory Th1 and Th17 cytokines in the skin. In addition, immune suppression was observed in the draining lymph nodes and spleens, such as suppression of Th1/Th17 cytokine expression. These suppression of cytokine expressions may be resulted from reduced numbers of Th17 cells in the lymph nodes and spleens. Recent discovery of suppressive effects of JTE-013 administration and *S1pr2* gene deficiency on T cell differentiation into Th17 and Th1 cells may explain the suppressed Th17/Th1 cytokine expression levels as shown in collagen-induced rheumatoid arthritis

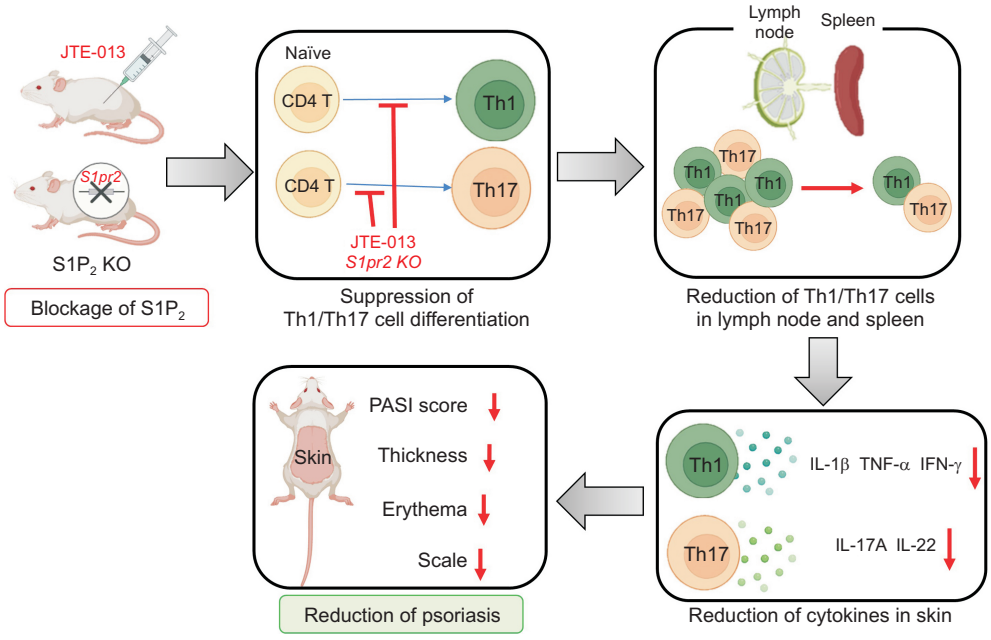

Fig. 9. Illustration of mechanism of action of JTE-013 treatment or *S1pr2* gene deficiency in psoriasis improvement. Administration of JTE-013 or *S1pr2* gene deficiency suppressed differentiation of naïve T cell into Th1 and Th17 cells, resulting in reduction of Th1 and Th17 cells in lymph nodes and spleen. Consequently, reduced production of Th1 and Th17 cytokines in the skin leads to improvement of imiquimod-induced psoriatic changes. PASI, Psoriasis Area Scoring Index.

model (Lee *et al*., 2024). Therefore, $S1P_2$ blockage may lead to suppression of Th17 and Th1 cell differentiation, which resulted in reduced numbers of Th17 and Th1 cells in the lymph nodes and spleens and reduced expression levels of Th17 and Th1 cytokines in the skin (Fig. 9). Consequently, reduced Th17 cytokines in the skin alleviate the psoriasis symptoms (Fig. 9).

Mast cells and Langerhans-like cells, which are dendritic cells in the skin, have been reported to express $S1P_1$, $S1P_2$, and S1P4 (Bock *et al*., 2016; Masuda-Kuroki and Di Nardo, 2022), although neutrophils do not express $S1P_2$ (Wilkins *et al*., 2023). Therefore, suppression of $S1P_2$ functions in mast cells and Langerhans cells may contribute to the therapeutic efficacy of JTE-013 and *S1pr2* deficiency in the present study. Similar immune suppressive effects of JTE-013 has been observed in atopic dermatitis-like and allergic asthma models by systemic administration and topical treatment (Park and Im, 2019, 2020; Kang *et al*., 2020). Suppressive effects of JTE-013 on activation of mast cells and maturation and migration of dendritic cells have been shown in both models (Park and Im, 2019, 2020; Kang *et al*., 2020). Although further studies on topical application of JTE-013 on psoriatic regions have to be conducted, the results suggest that $S1P_2$ may a beneficial target for psoriasis-like skin diseases.

Previously, S1P modulators targeting $S1P_1$ receptors, such as FTY-720 (fingolimod), IMMH002, and Syl930, have shown therapeutic potentials on psoriasis by suppressing inflammatory immune responses such as reduction in white blood cells, percentage of $CD3^+$ T cells, IL-23 mRNA levels in the blood, and IL-17A protein levels in the skin (Ji *et al*., 2018; Jin *et al*., 2020; Liu *et al*., 2021). $S1P_2$ blockage may have advantages to avoid the common side effects of $S1P_1$ modulator (fingolimod and ponesimod), such as, macular edema, atrioventricular conduction delays, bradyarrhythmias, and dyspnea (Mandal *et al*., 2017; Alnaif *et al*., 2023). However, because $S1P_2$ plays essential roles in development of neuronal excitability and functions of auditory and vestibular systems, cautions are needed to check the undesired side effects on neuronal, auditory, and vestibular functions during development of $S1P_2$ antagonist for psoriasis treatment. Therefore, it would be preferred to make a topical agent for psoriasis as in atopic dermatitis (Kang *et al*., 2020).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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AUTHOR CONTRIBUTIONS

JH Lee and DS Im: Designed the experiments; JH Lee: Performed the experiments and analyzed the data; DS Im: Wrote the manuscript.

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