Article

Discovery Medicine 2024; 36(189): 2026–2036
https://doi.org/10.24976/Discov.Med.202436189.186

BML-111 Modulates and Alleviates p38/MAPK Signaling Pathway and Th1/Th2/Th17 Cytokine Response in Murine Psoriasis-Like Dermatitis

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Published: 20 October 2024

Background: Psoriasis is a prevalent cutaneous inflammatory disorder characterized by elevated keratinocyte inflammation. 5(S)-6(R)-7-trihydroxyheptanoic-acid-methyl-ester (BML-111), an established analogue of lipoxin A4, is known for its potent anti-inflammatory properties. However, the precise role of BML-111 within a murine psoriasis-like dermatitis model requires further clarification. This research aims to investigate the modulatory effects of BML-111 on inflammatory responses, the p38/mitogen-activated protein kinase (MAPK) signaling cascade, and T helper type 1 (Th1), Th2, and Th17 cell responses within the context of a murine psoriasis-like dermatitis model.

Methods: A psoriasis-like dermatitis model was established by applying 5% imiquimod (IMQ) cream to the backs of C57BL/6 mice, which were pretreated intraperitoneally with or without BML-111 prior to IMQ application. Hematoxylin-eosin staining was utilized to detect the pathological alterations of the murine dorsal skin tissue. Furthermore, the psoriasis area and severity index (PASI) scoring system was used to assess the dynamic cutaneous alterations in the mice. The levels of tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), interleukin (IL)-1β, IL-6, IL-4, and IL-17A in the murine serum samples were quantified by means of enzyme-linked immunosorbent assays (ELISA). Western blotting was conducted to detect the proteins of TNF-α, IL-1β, IL-6, phospho-p38 (p-p38), and p38 in murine skin tissues. Lastly, a flow cytometry analysis was executed to evaluate the expression of peripheral blood Th1/Th2/Th17 cell subsets.

Results: BML-111 attenuated IMQ-induced pathological changes in skin tissue of psoriasis-like dermatitis mice. BML-111 treatment substantially reduced TNF-α, IL-1β, IL-6, IFN-γ and IL-17A levels and elevated IL-4 levels in serum and skin lesion tissues of IMQ-induced mice ($p < 0.01$, $p < 0.01$, $p < 0.01$, $p < 0.05$, $p < 0.05$, $p < 0.05$, respectively). The ratio of Th1/Th17 cells in the peripheral blood of BML-111-treated mice was substantially diminished and the ratio of Th2 cells was substantially augmented ($p < 0.05$, $p < 0.01$, $p < 0.001$, respectively). Mechanistically, p-p38 protein level was substantially reduced in the skin tissues of BML-111-treated mice ($p < 0.05$). While, dehydrocorydaline (DHC, a p38/MAPK pathway agonists) reversed the reduction of p-p38 protein level induced by BML-111 treatment in psoriasis-like mice ($p < 0.05$).

Conclusion: BML-111 modulates the p38/MAPK signaling pathway and Th1/Th2/Th17 cytokine response, and alleviates psoriasis-like dermatitis in mice.

Keywords: BML-111; psoriasis-like dermatitis; p38/MAPK signaling pathway; Th1/Th2/Th17 cytokine

Introduction

Psoriasis is a highly prevalent inflammatory skin condition characterized by a distinctive ruddy, scaling eruption. Microscopically, the psoriatic tissue exhibits an increase of epidermal cells exhibiting partial keratinization, along with penetration of several proinflammatory cells like lymphocytes and neutrophils into the dermis [1]. While the precise pathogenesis of psoriasis remains unclear, there is a prevailing belief that aberrant communication between keratinocytes and immune cells significantly contributes to the onset and progression of the condition [2]. In psoriasis, robust interactions between innate and adaptive immune cells, along with resident skin cells, appear to enhance and sustain chronic inflammation [3]. Therefore, strategies to alleviate excessive inflammation of keratinocytes have great potential for application in the treatment of psoriasis.

Lipoxin is an endogenous lipid mediator that has been proven to have a positive effect in inflammation resolution [4]. 5(S)-6(R)-7-trihydroxyheptanoic-acid-methyl-ester (BML-111), an analogue of lipoxin 4 but with a shortened C-7 and a common receptor, offers greater stability [5]. Previous studies have shown that BML-111 has anti-tumor, anti-inflammatory, and antioxidant stress effects. Recent *in vitro* studies have suggested that BML-111 may modulate the expression of inflammatory cytokines and chemokines, as well as influence the activation of immune cells implicated in psoriatic inflammation [6–8]. Further-

more, promising results in terms of BML-111's ability to attenuate psoriatic skin changes and reduce disease severity have been achieved with preclinical models [9]. These findings underscore the need for comprehensive *in vivo* studies to evaluate the therapeutic potential of BML-111 in the management of psoriasis. Another study investigated the improvement effect of BML-111 on psoriasis mice and reported the role of high mobility group box 1 (HMGB1) in it [10]. However, it is unclear whether BML-111 exerts its efficacy through other molecular mechanisms.

The p38/mitogen-activated protein kinase (MAPK) signaling cascade represents an integral pathway controlling diverse cellular functions such as inflammation and oxidative stress. The activation of this pathway has been detected in psoriatic lesions, playing a role in the development and progression of this dermatological disorder [11–13]. Prior investigations have demonstrated that suppressing inflammation via the inhibition of the p38/MAPK signaling cascade may represent a novel therapeutic strategy for treating psoriasis [14]. Besides, recent studies have highlighted the role of cytokines, particularly tumor necrosis factor alpha (TNF-α), interleukin (IL)-17, and IL-23, in driving the pathogenesis of psoriasis; therefore, targeting these cytokines presents a promising avenue for the development of novel therapeutic interventions against psoriasis [15,16]. Research has shown that the interaction between cytokines released by dendritic cells, T helper type 1 (Th1), Th2, and Th17 cells leads to the phenotype of psoriasis, and that therapies targeting cytokine-mediated psoriasis can improve the quality of life of patients [17].

This study aims to investigate the effects and potential mechanistic underpinnings of BML-111 in ameliorating psoriasis-like dermatitis and T cell differentiation incited by imiquimod (IMQ) by utilizing a murine model.

Materials and Methods

Experimental Animals

Twenty-four female C57BL/6 mice, aged approximately 6–8 weeks at procurement, were sourced from Beijing Biocisco Biomedical Technology Co., Ltd. (Beijing, China). These animals were housed within an environment maintained at a temperature of 22 $\pm$ 1 °C, a relative humidity of 45%–55%, and a light cycle consisting of 12:12 hour periods of light and darkness. Throughout the experiment, the mice were given access to food and water *ad libitum*. All experimental manipulations were conducted in strict accordance with approved animal ethics programs.

Construction and Grouping of Psoriasis Models

Twenty-four mice were randomly divided into four groups: normal, IMQ, and IMQ+BML-111, and IMQ+BML-111+dehydrocorydaline (DHC) groups, with 6 mice assigned to each group. At the beginning, the fur measuring an area of approximately 2 cm $\times$ 2 cm was removed from the back of each mouse. Except for the mice in the normal group, 5% IMQ cream (batch no. 18010340, Sichuan Mingxin Pharmaceutical Co., Ltd., Sichuan, China) was applied to the back of every mouse using a glass rod. The applied dose was 62.5 mg once/day for 6 days. The backs of mice in the normal group were coated with an equal amount of medical petroleum jelly (Item No. 20180130, Nanjing Turners Biotechnology Development Co., Ltd., Nanjing, China) daily. The mice in the IMQ+BML-111 and IMQ+BML-111+DHC subgroup were injected intraperitoneally with 1 mg/kg BML-111 (MCE, HY-100450, Shanghai, China) once/day for 6 days consecutively. The IMQ+BML-111+DHC group received simultaneous intraperitoneal injection of DHC (5mg/kg, HY-N0674, purity $\geq$98%, MCE, Shanghai, China) for 6 days consecutively. The mice in the normal and IMQ groups were injected intraperitoneally with an equal volume of saline. Eighteen mice of psoriasis models were successfully constructed. On the seventh day of the experiment, cervical dislocation of the experimental mice was conducted under anesthesia using 50 mg/mL of pentobarbital sodium (batch No. 080605, Shanghai General Regent Factory, Shanghai, China) for each animal. Skin tissues were excised from the back of each mouse. Bleeding from the eyes was induced before cervical dislocation, and the serum was stored in a cryogenic freezer set at a temperature of –80 °C.

Hematoxylin-Eosin (HE) Staining

Mouse skin tissues were routinely dehydrated and embedded in wax and then serially sectioned at a thickness of 3 µm Subsequently, the slides were stained in hematoxylin (Servicebio, G1004, Wuhan, China), differentiated with 1% hydrochloric acid ethanol, and stained in eosin staining solution (Solarbio, E8090, Beijing, China). After dehydration, the slides were cleared with xylene and sealed with neutral resin. Microscopic observation of the pathological skin damage by using a microscope (DM1000, Leica, Wetzlar, Germany) was performed in three randomly selected fields for each slide, and images were captured.

Evaluation of the Severity of Skin Inflammation

A systematic evaluation method, grounded in the application of clinical psoriasis area and severity index (PASI) score, was employed to gauge the intensity of skin lesions resembling psoriasis [18]. The rash was divided into grades 0–4, with 0 indicating no rash; 1 as mild; 2 as moderate; 3 as severe; and 4 as very serious. Animal weights and PASI scores were recorded daily.

ELISA Assay

Mouse serum was taken and the levels of inflammatory cytokines was detected. Enzyme-linked immunosorbent assays (ELISA) were performed to measure the levels of TNF-α (H052-1-2), IL-1β (H002-1-2), IL-6 (H007-1-

Table 1. Primer sequences for quantitative reverse-transcription polymerase chain reaction (qRT-PCR).

Gene	Sequences
Tumor necrosis factor alpha (*TNF-*α)	F: 5′-CCTGTAGCCCACGTCGTAG-3′ R: 5′-GGGAGTAGACAAGGTACAACCC-3′
Interleukin (*IL*)-*1*β	F: 5′-GAAATGCCACCTTTTGACAGTG-3′ R: 5′-TGGATGCTCTCATCAGGACAG-3′
IL-6	F: 5′-CTGCAAGAGACTTCCATCCAG-3′ R: 5′-AGTGGTATAGACAGGTCTGTTGG-3′
p38	F: 5′-ACCTAGCTGTGAACGAAGACT-3′ R: 5′-GTAGCCACGTAGCCTGTCATC-3′
Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*)	F: 5′-TGGCCTTCCGTGTTCCTAC-3′ R: 5′-GAGTTGCTGTTGAAGTCGCA-3′

2), interferon gamma (IFN-γ; H025-1-2), IL-4 (H005-1-2), and IL-17A (H014-2) in mouse serum. All ELISA kits were acquired from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The optical density (OD) value was measured utilizing an Infinite M200 spectrophotometer (Tecan, Morrisville, NC, USA) at a wavelength of 450 nm.

Quantitative Reverse-Transcription Polymerase Chain Reaction (qRT-PCR)

Total RNA was isolated using the TRIzol technique (15596018CN, Invitrogen, Carlsbad, CA, USA) and then reverse-transcribed using the PrimeScriptTM RT Reagent Kit (RR036A, Takara Biotechnology, Dalian, China) into cDNA, in strict adherence with the kit's specifications. QRT-PCR was conducted using the ABI7900 Real-time PCR system, employing the Sybr Premix Ex Taq Kit (RR820A, Takara Biotechnology, Dalian, China). In this experiment, 40 cycles of amplification were set, and the $2^{-\Delta\Delta CT}$ method was used to calculate the relative gene expression. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as an internal reference, and the sequences of primers are displayed in Table 1.

Western Blotting

After sacrifice, skin tissues was corrected from the back of each mouse using scalpels (Shanghai Zhiheng Medical Device Co., Ltd., Shanghai, China). Total proteins were isolated using radio-immunoprecipitation assay (RIPA) lysis buffer (R0010, Solarbio, Beijing, China), and their concentrations were measured using a bicinchoninic acid (BCA) kit (PC0020, Solarbio, Beijing, China). To separate the extracted proteins, a 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) technique was employed by using 20 µg of proteins per well. The separated proteins were subsequently transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, IPVH00010, MA, USA). The membranes were blocked with 5% skimmed milk solution at 4 °C. Primary antibodies targetting TNF-α (1:500, ABclonal, A20851, Boston, MA, USA), IL-1β (1:500, ABclonal, A16288, Boston, MA, USA), IL-6 (1:500, ABclonal, A0286, Boston, MA, USA), phospho-p38 (p-p38, 1:500, ABclonal, AP0526, Boston, MA, USA), p38 (1:500, ABclonal, A14401, Boston, MA, USA), GAPDH (1:500, ABclonal, AC001, Boston, MA, USA) were separately added for a 12-hour incubation with the membranes. The membranes were then subjected to a one-hour incubation with goat anti-rabbit IgG secondary antibody (1:1000, Solarbio, K1034G-AF594, Beijing, China). Subsequently, the enhanced chemiluminescence (ECL) Kit (Thermo Fisher Scientific, WP20005, Shanghai, China), coupled with the Bio-Tanon imaging apparatus (5200, Bio-Tanon, Shanghai, China), was employed for visualization. Image analysis and protein quantification were conducted using the Image J software (Version 1.8.0, Media Cybernetics, Silver Spring, MD, USA), employing gray scale analysis methodology.

Flow Cytometry

The blood specimens collected from the mouse eyeballs were anticoagulated with heparin. Lymphocyte layer was obtained by separating the blood specimens using lymphocyte separation solution. After washing with phosphate buffer saline (PBS), the cells were resuspended in Roswell Park Memorial Institute (RPMI)-1640 (11875119, Gibco, Carlsbad, CA, USA). The cell suspension was incubated with 2% trypan blue staining solution (T8070, Solarbio, Beijing, China) for 5 min and placed in a cell counter (C100-SE/C100, RuiWoDe, Shenzhen, China) for cell count analysis. This enriched lymphocyte suspension was transferred into a 24-well tissue culture plate. A total of 2×10^6 cells were dispensed into each well. Phorbol myristate acetate (PMA; P8139, Sigma-Aldrich, Saint Louis, MO, USA) at a final concentration of 50 ng/mL, ionomycin (I3909, Sigma-Aldrich, Saint Louis, MO, USA) at a final concentration of 1 µg/mL, and monensin (475895, Sigma-Aldrich, Saint Louis, MO, USA) at a final concentration of 2 mmol/L were added. The cells were then cultivated under optimal conditions of 37 °C and 50% CO_2 for a period of 4 hours, after which they were harvested. Post-harvest, the cells underwent two rounds of PBS washing

and then incubated with fluorescein isothiocyanate (FITC)-labeled anti-mouse CD3 (MHCD0301, Thermo Fisher Scientific, Shanghai, China) and PE-labeled anti-mouse CD4 antibodies (MCD0417, Thermo Fisher Scientific, Shanghai, China) in dark at 4 °C for 30 min. After washing twice, 100 mL of the fixative was added. After fixation at 4 °C for 15 min, the membrane-breaking solution was added and incubated at 4 °C for 15 min. After washing twice, each tube was divided into 3 parts and incubated with APC-labeled anti-mouse IFN-γ, IL-4, IL-17A antibodies (Thermo Fisher Scientific, MHCIFG05, 17-7041-81, 17-7177-81, Shanghai, China) in the dark at 4 °C for 30 mins. Following another round of washing, the cells were subjected to flow cytometric analysis (CytoFLEX S, Beckman Coulter, San Jose, CA, USA) to determine the proportions of the cell population.

Statistical Analysis

Data analysis was executed utilizing the IBM SPSS 22.0 statistical package (IBM SPSS Inc., Chicago, IL, USA). Results are presented in mean $\pm$ standard deviation (SD), and the differences between two groups were analyzed using Student's *t*-tests. Intergroup variances were assessed via one-way analysis of variance (ANOVA), coupled with subsequent post-hoc procedures (Tukey's method). Significance was interpreted at a threshold of $p < 0.05$.

Results

BML-111 Improved IMQ-Induced Psoriasis-Like Dermatitis in Mice

HE staining was performed to better evaluate morphological alterations in murine dorsal skin tissue. These examinations indicated that the epidermis and dermis components in the skin tissues of the normal group exhibited regular architecture, maintaining their normal histological patterns. Significant epidermal hyperplasia, thickening of the spinous cell layer, and incomplete keratinization were observed in mouse skin tissue sections treated with IMQ, with a large number of inflammatory cells infiltrating the dermis. Pre-treatment with BML-111 could alleviate the histological damage caused by IMQ, marked by relatively flat epidermal layer, and substantially reduce number of cells with incomplete keratinization (Fig. 1A). Furthermore, the epidermis of the IMQ-treated mice significantly grew in thickness when compared to the control group ($p < 0.001$), whereas pre-treatment with BML-111 drastically diminished the epidermal thickness as opposed to the mice solely exposed to IMQ ($p < 0.01$) (Fig. 1B). Similarly, the PASI score exhibited by the IMQ group markedly surpassed that of the control group ($p < 0.001$), but it was mitigated in the IMQ+BML-111 group ($p < 0.01$) (Fig. 1C). On the sixth day, the body weight of IMQ-treated mice notably declined ($p < 0.001$); however, administration of BML-111 instigated an increase in body weight when compared to the IMQ group ($p < 0.05$) (Fig. 1D). Furthermore, spleen weight was considerably elevated in the IMQ group when compared to the control group ($p < 0.01$), but it reduced upon IMQ+BML-111 treatment ($p < 0.05$) (Fig. 1E).

Effect of BML-111 on IMQ-Induced Psoriasis-Like Skin Inflammation in Mice

ELISA data revealed marked increases in the serum levels of TNF-α, IL-1β, and IL-6 among mice of the IMQ group when compared to those of the normal group ($p < 0.001$). Remarkably, a reduction in these cytokine levels was observed in the IMQ+BML-111 group ($p < 0.01$) as compared to the IMQ group (Fig. 2A). *In vitro* experiments employing qRT-PCR and Western blotting techniques demonstrated significant upregulation of TNF-α, IL-1β, and IL-6 mRNA and proteins in skin tissues of the IMQ group relative to the normal group ($p < 0.001$). Conversely, a substantial decline in these protein levels was significant in the IMQ+BML-111 group when compared to the IMQ group ($p < 0.05$) (Fig. 2B,C). Collectively, these findings underscore the ability of BML-111 to effectively mitigate the development of psoriasis-like skin inflammation triggered by IMQ in murine models.

Effect of BML-111 on IMQ-Induced Th1/Th2/Th17 Cytokine Response in Mice

In contrast with the normal group, the proportion of Th1/Th17 cell subsets in the peripheral blood of mice in the IMQ group was substantially augmented, and the proportion of Th2 cell subsets was substantially diminished ($p < 0.01$). In contrast with the IMQ subgroup, the IMQ+BML-111 group exhibited significantly diminished proportion of Th1/Th17 cell subsets and augmented proportion of Th2 cell subsets in peripheral blood, which were validated by flow cytometry ($p < 0.05$) (Fig. 3A). According to the ELISA results, when compared to the control group, the serum levels of IFN-γ and IL-17A of the IMQ cohort displayed a remarkable increase, concurrently with a marked decrease in the level of IL-4 ($p < 0.01$). Upon administration of the BML-111, a statistically significant reduction in the serum levels of IFN-γ and IL-17A was observed, accompanied by an elevated IL-4 level ($p < 0.05$), as depicted in Fig. 3B.

Effect of BML-111 on p38/MAPK Signaling Pathway in IMQ-Induced Psoriasis-Like Dermatitis in Mice

The analysis of qRT-PCR displayed that the p38 expression level in IMQ group was up-regulated compared to the normal group ($p < 0.01$), and BML-111 down-regulated the p38 expression level in IMQ-induced psoriasis mice ($p < 0.05$) (Fig. 4A). The Western blotting results showed that p-p38 protein level in the skin tissue of the IMQ group was substantially augmented compared to that the normal group ($p < 0.01$). Contrary to the IMQ group, the IMQ+BML-111 group demonstrated significantly reduced protein level of p-

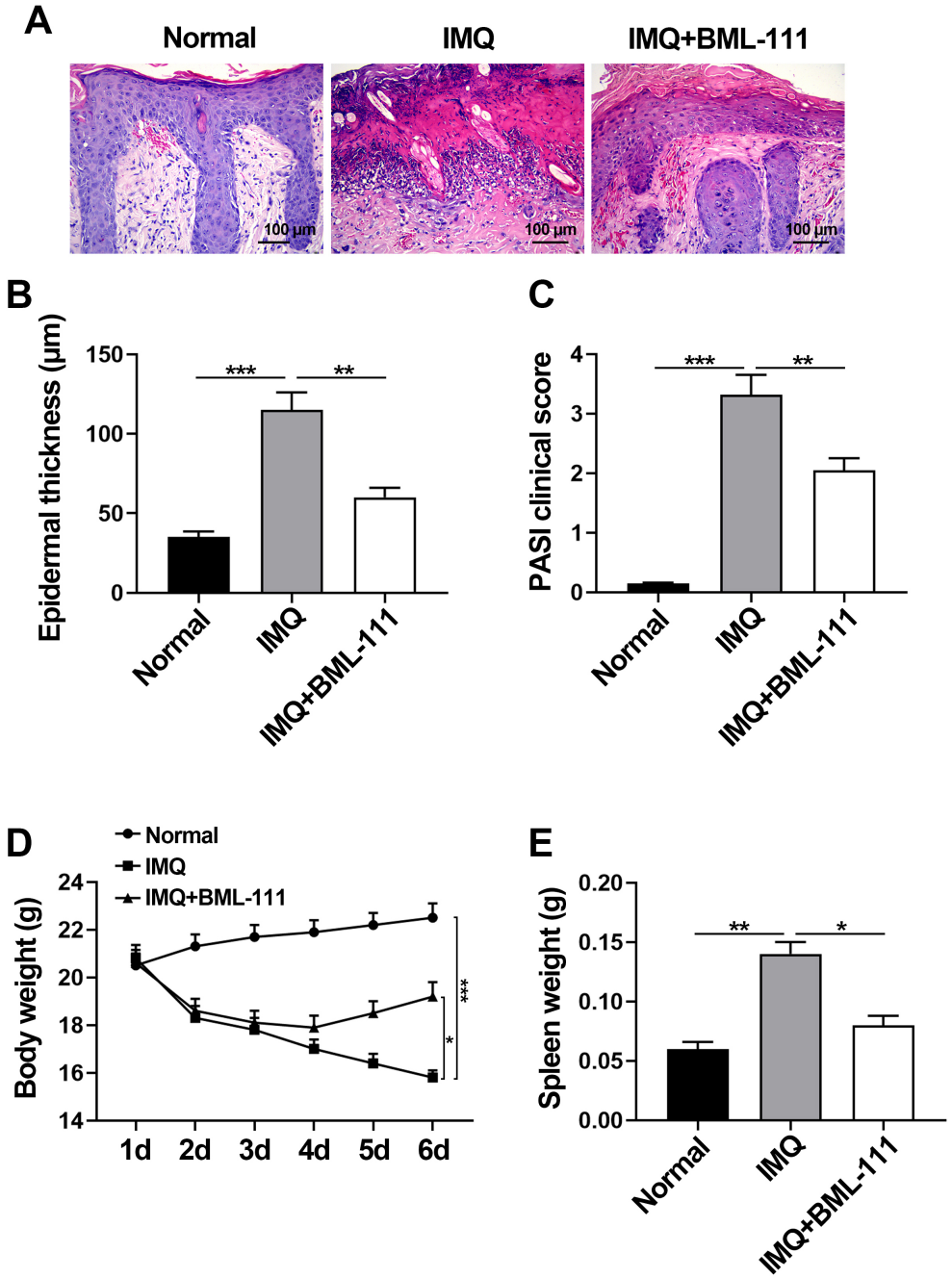

Fig. 1. 5(S)-6(R)-7-trihydroxyheptanoic-acid-methyl-ester (BML-111) ameliorated imiquimod (IMQ)-induced psoriasis-like dermatitis in mice. (A) Hematoxylin-eosin (HE) of the back skin cross-section of mice. (B) Epidermal thickness of the back skin. (C) Psoriasis area and severity index (PASI) score. (D) Body weight of mice. (E) Spleen weight. n = 6. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

p38 in the skin tissue ($p < 0.05$) (Fig. 4B). To elucidate the mechanism underlying the BML-111-mediated alleviation of IMQ-induced psoriasis-like dermatitis in mice, we intraperitoneally administered DHC (a p38/MAPK pathway agonist) to the mice. Analysis by qRT-PCR suggested that p38 expression level in IMQ+BML-111+DHC group was up-regulated compared with IMQ+BML-111 group ($p < 0.05$) (Fig. 4C). Western blotting demonstrated the p-p38 protein expression in IMQ+BML-111+DHC group was increased compared with IMQ+BML-111 group ($p < 0.05$) (Fig. 4D). The ELISA results revealed that, relative to the IMQ group, the serum levels of TNF-α, IL-1β, IL-6, IFN-γ, and IL-17A of the mice in the IMQ+BML-111 group were decreased ($p < 0.05$), while a significant increase in IL-4 level was detected ($p < 0.05$). This pattern of cytokine levels was reversed in the IMQ+BML-111+DHC group ($p < 0.05$) (Fig. 4E,F).

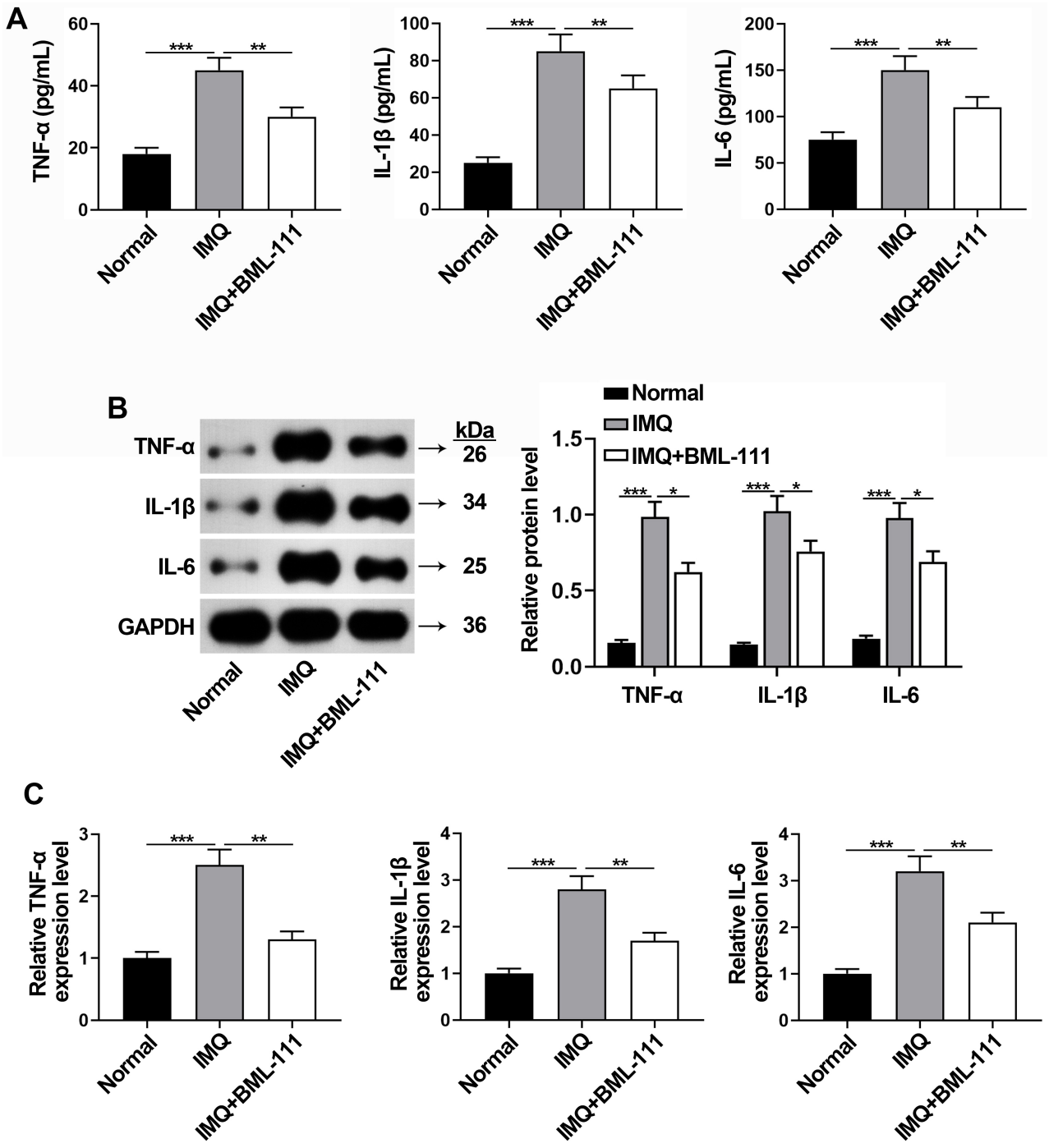

Fig. 2. Effect of BML-111 on IMQ-induced psoriasis-like skin inflammation in mice. (A,B) Levels of TNF-α, IL-1β and IL-6 in mice as detected by means of enzyme-linked immunosorbent assays (ELISA) (A) and Western blotting (B). (C) Expression of *TNF-α*, *IL-1β* and *IL-6* in mouse skin tissue measured by means of qRT-PCR. n = 6. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Discussion

In the current study, we observed that a BML-111 treatment resulted in a marked reduction in the severity of skin abnormalities and diminished inflammation in murine IMQ-induced psoriasis, orchestrated primarily through the regulation of the p38/MAPK signaling cascade and the Th1/Th2/Th17 cytokine profile.

IMQ has been demonstrated to induce dermatitis in mice, which closely resembles human psoriasis. Consequently, the IMQ-induced psoriatic murine model has gained significant recognition in scientific research [19]. Consistent with prior findings, our present study also revealed that IMQ-treated mice displayed several characteristics consistent with psoriatic lesion development. HE staining confirmed significant epidermal hyperplasia, thicken-

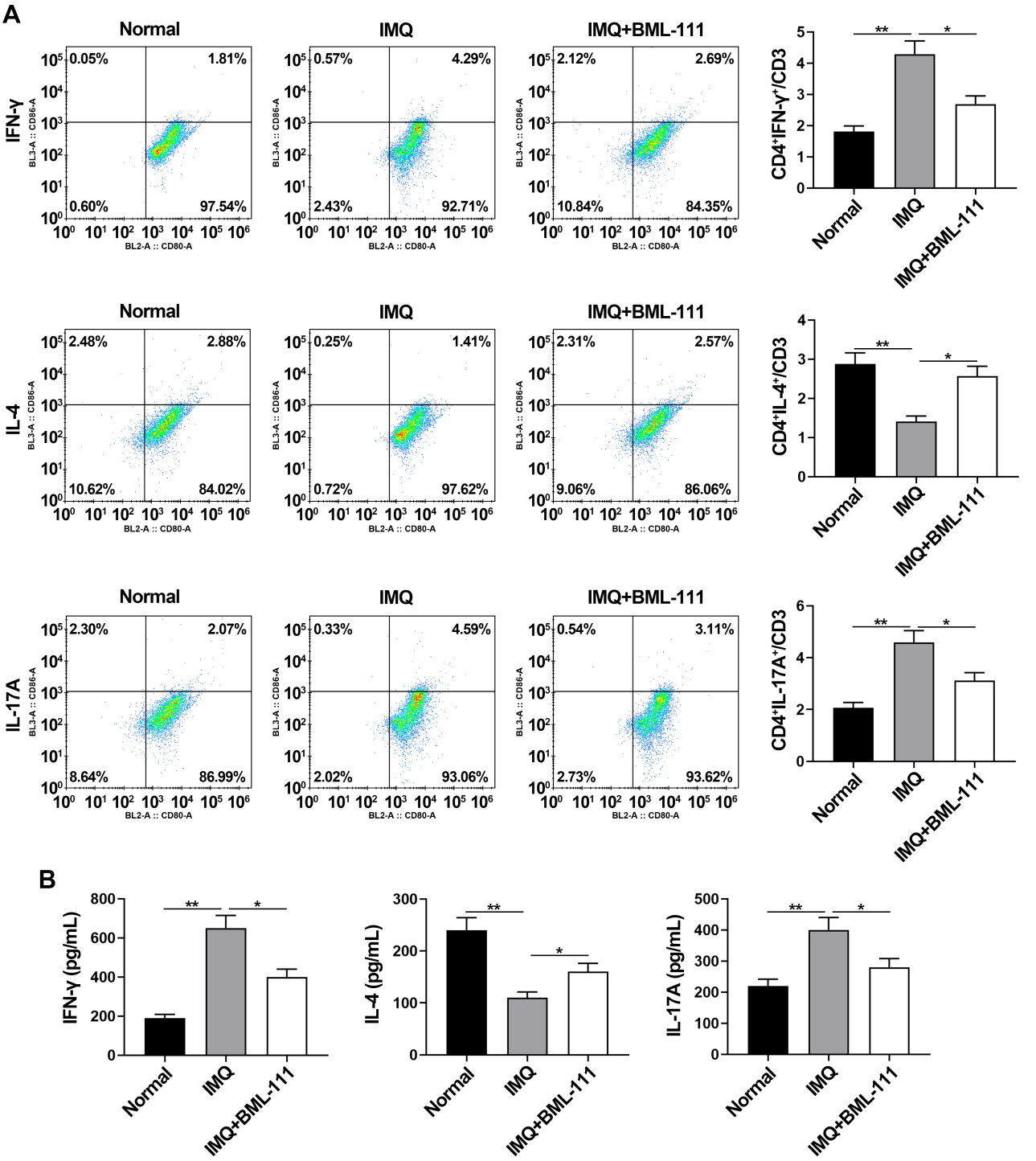

Fig. 3. Effect of BML-111 on IMQ-induced Th1/Th2/Th17 cytokine response in mice. (A) Flow cytometric detection of peripheral blood T helper type 1 (Th1)/Th2/Th17 cell subsets. (B) Detection of serum interferon gamma (IFN-γ), IL-4 and IL-17A by means of ELISA. n = 6. *$p < 0.05$, **$p < 0.01$.

ing of the spinous cell layer, and incomplete keratinization, as well as extensive inflammatory cell infiltration. In addition, IMQ induced significant thickening of the mouse epidermis and a significant increase in PASI score. Thus, treatment with BML-111 can dramatically alleviate IMQ-induced psoriasis-like dermatitis, improve its histopathological features and epidermal thickness, and dramatically reduce the PASI score. In light of its alleviative effect on IMQ-induced skin damage, BML-111 may serve as a strategy for treating psoriasis-like dermatitis, as indicated in these results.

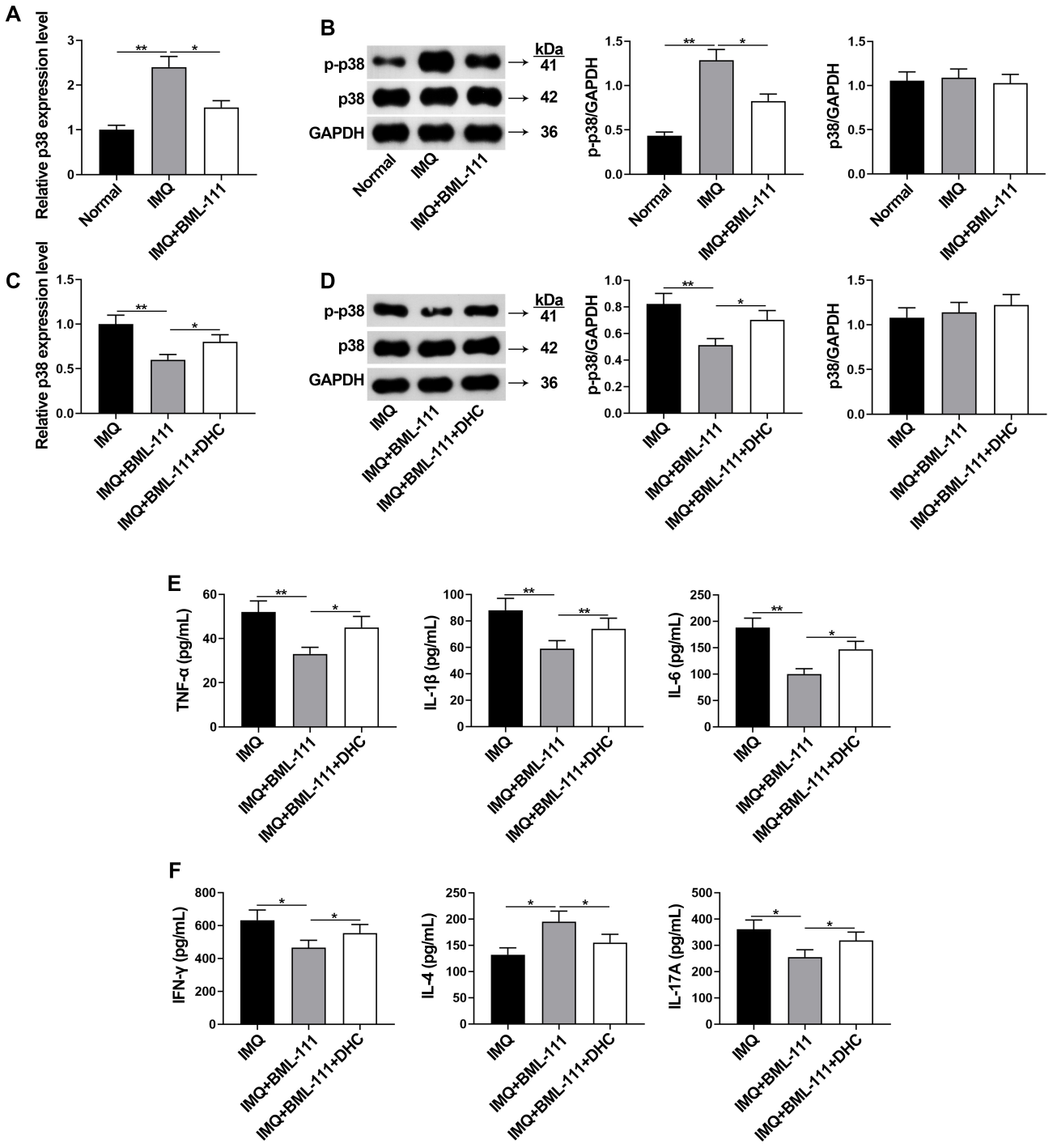

Fig. 4. Effect of BML-111 on p38/mitogen-activated protein kinase (MAPK) signaling pathway in murine IMQ-induced psoriasis dermatitis. (A) qRT-PCR detection of the *p38* mRNA expression in the mouse skin tissues of each group. (B) Western blotting detection of phospho-p38 (p-p38) and p38 protein levels in mouse skin tissues of each group. (C) Quantification of *p38* mRNA expression in mouse skin tissues of each group by qRT-PCR. (D) Detection of p-p38 and p38 protein levels in mouse skin tissues of each group by means of Western blotting. (E) Serum levels of TNF-α, IL-1β and IL-6 of each group. n = 6. (F) Serum levels of IFN-γ, IL-4 and IL-17A of each group. n = 6. *$p < 0.05$, **$p < 0.01$. DHC, dehydrocorydaline.

The pivotal roles of TNF-α, IL-1β, and IL-6—the pivotal pro-inflammatory cytokines—in inducing and exacerbating the psoriasis inflammatory cascade have been demonstrated in several studies [20,21]. Notably, it has been established that TNF-α can stimulate the NF-κB signaling pathway, thereby contributing significantly to the development of psoriasis. Presently, TNF-α antagonists have gained widespread acceptance within the realm of clinical

management for psoriasis [17,22]. Tang *et al.* [23] demonstrated that effective inhibition of abnormal upregulation of IL-1β in keratinocytes and subsequent IL-1β-mediated inflammatory cascades can alleviate psoriasis-like symptoms. A study by Cai *et al.* [24] confirmed that inhibiting the expression of TNF-α and IL-6 can effectively improve the characteristic lesions of psoriasis in mice. Our study found that the serum levels of TNF-α, IL-1β and IL-6 and the dorsal skin lesions were dramatically augmented in the IMQ group compared to the normal group, while BML-111 treatment dramatically reduced the expression of these inflammatory cytokines. This suggests that BML-111 can alleviate IMQ-induced skin inflammation. Liu *et al.* [10] analyzed the changes of these inflammatory factors in skin samples of psoriasis mice treated with BML-111 and found similar changes to ours.

Dendritic cells/IL-23/Th17 cells/keratinocyte axis is key to promoting psoriasis progression and positive feedback loop [25]. In a study to comprehensively examine the function of Th lymphocytes within psoriasis, Hu *et al.* [26] found that both infectious agents and physical trauma serve as stimuli for dendritic cells to secrete IL-23, TNF-α, and IL-12, which in turn trigger the IL-23 and/or IL-22 cascade, induce maturations of Th17 and/or Th22 cells, and generate an abundance of cytokines that impact keratinocytes and exacerbate inflammation in the context of psoriasis. IL-17A is produced by Th17 and promotes the proliferation of epidermal keratinocytes. In addition, IL-17A upregulates chemokines produced by keratinocytes [27]. The drugs that block IL-17A were approved for psoriasis treatment, including secukinumab and ixekizumab [17]. Yan *et al.* [28] confirmed that the downregulation of the Th1/Th17 cell ratio can reduce IMQ-induced psoriasis-like inflammation in mice. Our investigation indicated that IMQ could raise the ratio of Th1/Th17 lymphocytes in mouse peripheral blood, diminish the proportion of Th2 cells, enhance the levels of IFN-γ and IL-17A present in mouse serum, and reduce the level of IL-4. In contrast, treatment with BML-111 reduced the ratio of Th1/Th17 lymphocytes, elevated the proportion of Th2 cells, and diminished the levels of IFN-γ and IL-17A in mouse serum while increasing IL-4 levels. These findings suggest that the administration of BML-111 may alter the generation of Th cells and their secretion of cytokines in mice, thereby mitigating the manifestations of psoriasis.

The p38/MAPK signaling cascade modulates an array of cellular activities, encompassing proliferation and inflammation [29]. According to Jiang *et al.* [12], p38/MAPK signaling pathway is activated in psoriasis and involved in immune regulation. In this study, we found that following the induction of psoriasis-like dermatitis using IMQ, p-p38 protein level in mouse skin tissue was elevated, which could be attenuated using a BML-111 treatment. To further confirm whether the effect of BML-111 on IMQ-induced psoriasis-like dermatitis is related to the p38/MAPK signaling pathway, mice were treated with BML-111 in combination with DHC, a p38/MAPK signaling pathway agonists. In contrast with the mice treated with BML-111 alone, those receiving the combined treatment exhibited an increase of p-p38 protein level in their skin tissue. Of note, *p38* mRNA expression, instead of p38 protein expression, manifested significant differences among the groups. This may be caused by inconsistencies in gene transcription and translation levels. From a gene to a functional protein, a series of processes such as transcription, translation, and post-translational modifications are required to encode a protein, and these processes are subject to spatial and temporal regulations by multiple factors. The similar discordant expressions of mRNAs and their corresponding proteins have been reported in previous studies, which attributed such phenomenon to the potential influence of multiple factors such as post-translational modifications and degradation of proteins [30,31]. The potential factors underlying the inconsistent expression of p38 protein and mRNA in this study require further in-depth investigations. In addition, we found that TNF-α, IL-1β, IL-6, IFN-γ and IL-17 A protein levels in the skin tissues of mice receiving the combined treatment were further reduced, and IL-4 protein level demonstrated an opposite trend, *i.e.*, a further increase. This result indicates that BML-111 can reduce the inflammatory response triggered by IMQ through the regulation of p38/MAPK signaling pathway. Our study did not delve into the changes of downstream cascade signals of p38 induced by BML-111, which would be further analyzed in future research.

Conclusion

In summation, BML-111 modulates the p38/MAPK signaling pathway and Th1/Th2/Th17 cytokine response, reduces the release of proinflammatory factors, and improve IMQ-induced psoriasis-like dermatitis in mice. Thus, BML-111 holds promise as a potential agent for psoriasis treatment.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Author Contributions

QinZ and YA designed the research. YA and QioZ performed the experiments. YR provided help and advice on the flow cytometry. YR and SY analyzed the data. YA and QinZ jointly wrote the first draft. All authors contributed significantly to editorial changes of important content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

According to the Animal Ethics Procedures and approved by the The First Affiliated Hospital of Heilongjiang University of Chinese Medicine Animal Ethics Committee (Ethical clearance No. HZYDWLLKY202311033). All animal experiments were performed according to the Animal Ethics Procedures.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

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