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Original Research

Effects of Imiquimod Application Durations on Psoriasis-like Lesions and Cytokine Expression in Mice

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ABSTRACT

Introduction: Psoriasis is a chronic inflammatory skin disorder with a complex pathogenesis, prominently involving the IL-17/IL-23 pathway. The imiquimod (IMQ)-induced mouse model is widely utilized for preclinical psoriasis research. This study aims to elucidate the effects of different durations of IMQ exposure on the development and characteristics of psoriasis-like lesions and the underlying changes of related cytokines in mice. **Methods**: *Balb/c* mice were topically administered IMQ on their shaved dorsal skin for either six or twelve consecutive days to induce psoriasis-like lesions, with untreated mice serving as controls. Clinical symptoms were assessed, and the expression levels of IL-17A and IL-23 were quantified using RT-PCR and immunohistochemistry. Histopathological changes were evaluated through H&E staining. **Results**: Mice exposed to IMQ displayed significant psoriasis-like lesions, with peak severity observed on day six across both exposure groups. Long-term exposure (12 days) did not aggravate the severity of lesions but affected the dynamics of cytokine expression and histopathological features. Notably, IL-17A and IL-23 levels correlated with the progression and severity of lesions in the short-term exposure group, decreasing significantly after the cessation of IMQ application. **Conclusion**: Short-term IMQ exposure (six days) effectively induces psoriasis-like lesions in mice, highlighting the pivotal role of the IL-17/IL-23 axis in this model. Extended exposure to IMQ (twelve days) offers no additional benefit in terms of lesion severity, suggesting that a six-day protocol is optimal for establishing a mouse model of psoriasis for the evaluation of therapeutic interventions targeting the IL-17/IL-23 pathway.

Key words: IL-17/IL-23 pathway, imiquimod, inflammatory skin disorders, psoriasis, preclinical models, psoriasis research

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INTRODUCTION

Psoriasis is more than just a common skin condition—it's a complex chronic disease that affects approximately 2-3% of the global population. This disorder originates from an intricate interplay of immune system dysfunctions, primarily involving T cells and cytokines interleukin-17 (IL-17) and interleukin-23 (IL-23), which play crucial roles in the development and severity of psoriasis symptoms[1]. The condition is notably visible, presenting as red, scaly patches that can cover extensive areas of the body and scalp, along with nail issues such as pitting, discoloration, and thickening. Furthermore, about one-third of those affected will experience joint damage, leading to significant physical deformities and a decrease in their quality of life[1]. The impact of psoriasis extends beyond the skin, with associations with serious health conditions like cardiovascular diseases, metabolic syndromes, and mental health issues, notably depression. These associations contribute to a heightened risk of premature death among sufferers[1].

Despite the development of more effective therapies, including biological treatments targeting IL-17A and IL-23[2–4], a permanent cure or long-term remission remains elusive, with concerns over side effects, potential infections, and the specter of drug resistance lingering, underscoring the urgent need for research into more effective and sustainable treatment options. The quest for better treatments hinges on a deeper understanding of the disease's underlying immune mechanisms.

Preclinical studies using animal models that replicate the clinical and pathological features of psoriasis are crucial for evaluating the potential of new therapeutic agents. Numerous models of psoriasis have been investigated, encompassing direct induction, spontaneous induction, genetic manipulation, and xenotransplantation[5,6]. One such example is the spontaneous mouse model, characterized by genetically altered mice displaying the hallmark red, scaly skin of psoriasis but lacking T-cell infiltration. However, the xenograft and genetic engineering models demand substantial technical proficiency and financial investment. Notably, the imiquimod (IMQ)-induced

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psoriasis-like mouse model stands out among these approaches for its accessibility, cost-effectiveness, and faithful replication of clinical and histopathological aspects of the disease.

IMQ, a Toll-like receptor (TLR) 7 agonist, not only provides a practical method for inducing psoriasis-like conditions in mice but also offers valuable insights into the role of the IL-17/23 pathway in psoriasis pathogenesis[7,8]. Interestingly, this model has demonstrated that inhibiting IL-17A can significantly reduce psoriasis-like symptoms in mice, underscoring the importance of this pathway in the disease[7,9]. Our research seeks to enhance the utility of the IMQ-induced psoriasis-like model by investigating the effects of both short-term and prolonged IMQ application on the development and persistence of psoriasis-like features in mice. Although the effectiveness of short-term IMQ application is well-established, there is limited understanding of the consequences of prolonged exposure, thus underscoring the significance of this study. This approach aims to fill a significant gap in existing research, providing fresh insights into the suitability of the model for assessing long-term therapeutic strategies. By doing so, we hope to increase the model's relevance and applicability in the continuous effort to understand and effectively manage psoriasis.

METHODS

Animals

Male and female mice aged 8–11 weeks were obtained from the Laboratory for Animal Care and Use, Stem Cell Institute, University of Science, Vietnam National University, Ho Chi Minh City (HCMUS), Viet Nam. The mice were housed in a 12-hour light/dark cycle and provided with ad libitum access to food and water. They were allowed to acclimate for 7 days before the commencement of the experiments. All animal procedures were approved by the Animal Ethics Committee of the Stem Cell Institute, University of Science, Viet Nam National University, Ho Chi Minh City, Viet Nam (220102/SCI-ACE), and were conducted following institutional guidelines.

The allocation of animals to treatment groups, the encoding of labels for experimental groups, and all subsequent handling were conducted by personnel not directly involved in data analysis.

Imiquimod-Induced Psoriasis-Like Model

The dose of IMQ cream (3.125 mg) and the duration of IMQ application for 6 days were selected based on previous studies and established protocols in the literature[7,10–12]. This dosage has been widely utilized in preclinical research to induce psoriasis-like lesions in mouse models effectively.

The dorsal skin of each mouse was shaved over an area of 4 cm^2 (2.0 x 2.0 cm). After a 48-hour observation period, mice were randomly divided into three groups (n = 30). A dose of IMQ 3.125 mg (Imiquad^{TM}; Glenmark) was applied to the shaved skin area to induce psoriasis-like lesions for either 6 days or 12 days consecutively. Shaved normal mice without IMQ application served as controls.

Clinical Assessment

Erythema, scaling, and thickness were evaluated based on the modified Psoriasis Area and Severity Index (PASI). Each parameter was independently scored on a scale from 0 to 4, representing no clinical signs (0), slight (1), moderate (2), marked (3), and very marked (4) clinical signs. The sum of these three features was calculated to determine the severity of the lesion, ranging from 0 to 12 [7].

Measurement of Skin Thickness

Skin thickness was measured at three separate positions in the experimented area on the back using a Mitutoyo electronic caliper (Mitutoyo, America) every 24 hours. The difference in back skin thickness was calculated as the average thickness on the following day minus that on the first day.

Histological Analysis

Mice were anesthetized with zolazepam, tiletamine (Zoletil 50, Virbac, India), and xylazine hydrochloride (XYL-M2, Inovet, Belgium) via intramuscular injection. Dorsal skin samples (5.0 x 5.0 mm) at the experimented sites were collected, fixed in 4% paraformaldehyde for 2-3 hours, and processed for hematoxylin and eosin (H&E) staining. Histopathological features were assessed on days 3, 6, 12, and 24 using the Psoriasis Histopathology Score (PHS).

The Psoriasis Histopathology Score (PHS) was calculated based on the components of inflammatory cell infiltration, parakeratosis, acanthosis, epidermal thickness, and Munro's abscess (with grades ranging from 0 to 3) (0 = none, 1 = slight, 2 = moderate, and 3 = marked)[10]. An Axiovert microscope and AxioVision software (Zeiss, Germany) were utilized to photograph H&E images of each slide and measure epidermal thickness on H&E images. For each skin sample, three slides were taken, and on each slide, three random areas were selected to measure epidermal thickness.

The clinical expression of mice and the histological structure of tissue were evaluated by two independent

researchers. Subsequently, the results were validated by blinded dermatologists and pathologists who were unaware of the treatment groups.

Real-Time Quantitative PCR

Tissue samples were obtained from the investigated areas and stored at -86°C until further processing. Total mRNA was extracted using the Easy-BLUE™ Total RNA Extraction Kit (iNtRON Biotechnology, Korea), and real-time quantitative RT-PCR analysis was performed to measure the mRNA levels of IL-17A, IL-23, and GAPDH. Sequences for the PCR primers are listed in Table I. Gene expression levels were normalized to GAPDH using the Livak and Schmittgen method [13–15].

Immunohistochemistry

Skin specimens were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned. Sections underwent deparaffinization and were then incubated with primary antibodies targeting IL-23p19 and IL-17A (Thermo Fisher Scientific, Waltham, MA, USA) followed by secondary antibody incubation. Nuclei were stained with Hoechst 33342 (Sigma-Aldrich, USA). Immunohistochemistry images were captured using a Zeiss Confocal Observer Z1 Laser Scanning Microscope (Zeiss, Germany) for qualitative analysis.

Statistical Analysis

Data were analyzed using SPSS 20.0 and GraphPad Prism software. Descriptive statistics were presented as mean ± standard deviation or median (minimum-maximum) for quantitative variables. The Student's t-test and Mann-Whitney U test were employed for comparisons between groups. Significance was set at $p < 0.05$.

RESULTS

Imiquimod-induced Psoriasis-like Clinical Features

The normal mice displayed a healthy complexion, smooth skin texture, and thin skin with an average skin thickness of 21.61 ± 1.24 mm. Following shaving, the fur of mice did not regenerate within the 12-day observation period (**Figure 1** A1-H1). Upon administration of IMQ, both the IMQ 6D (**Figure 1** A2-H2) and IMQ 12D groups (**Figure 1** A3-H3) exhibited well-defined plaques on the treated area of the dorsal skin with bright red erythema, numerous white scales, and increased skin thickness on day 3 (Fig. 1 B2, B3), peaking on day 6 (**Figure 1** C2, C3). Notably, in the IMQ 12D group, despite the continued application of IMQ, there was a slight decrease in the psoriasis characteristics mentioned above (**Figure 1** D3-E3). After discontinuation of IMQ, the IMQ 6D group showed a reduction in erythema, scales, and thickness by day 8 (**Figure 1** D2). From the 12^{th} to the 24th day, no scales were observed on the skin, with a slight skin thickening (**Figure 1** E2-H2). On the 24^{th}, all mice had no clinical symptoms on their skin (**Figure 1** H2). In the IMQ 12D group, the severity of scales and erythema significantly decreased on day 14 (**Figure 1** F3). On day 24, the investigated areas on the back skin lacked erythema and scales (**Figure 1** H3). In normal mice, the PASI score remained at 0 throughout the experimental period. However, in the IMQ 6D group, during the application of IMQ, scores for skin thickening, scaling, and erythema significantly increased from day 3, peaking on day 6 with scores of 3.43 ± 0.51, 3.83 ± 0.38, and 3.30 ± 0.47, respectively. Similar findings were seen in the IMQ 12D group (**Figure 2** A, B, C). The PASI score on day 6 reached 10.57 ± 0.77, indicating a statistically significant increase compared to the normal skin, and this elevation persisted throughout the 12 days of IMQ application (**Figure 2** D). Notably, individual component scores fluctuated during this period. On day 12, the redness score significantly decreased to 2.93 ± 0.25, and the scale score gradually decreased to 3.3 ± 0.47 (**Figure 2** B, C). In contrast, the skin thickness score remained prominent from day 6 to day 12 with a score of 3.7 ± 0.47, significantly higher compared to the normal group ($p < 0.05$) (**Figure 2** A). Upon discontinuation of IMQ treatment, both the PASI score and its component scores for scales, erythema, and thickness gradually decreased (**Figure 2**). In the IMQ 6D group, this reduction was most pronounced on day 8, with PASI scores, erythema scores, scaling scores, and skin thickness being 5.27 ± 0.64, 1.8 ± 0.4, 1.5 ± 0.5, and 1.97 ± 0.18, respectively. By day 18, both the scale score and erythema score were 0 (**Figure 2** B, C), but the investigated skin was still slightly thick (**Figure 2** A). The thickness score of the affected skin decreased to 0 on day 24 (**Figure 2** A). Regarding the IMQ 12D group, the erythema and scaling scores rapidly decreased to 0 by day 18; however, the thickness score still maintained 1 point on day 24, significantly higher than that of the normal group (**Figure 2** A, B, C).

Histopathological Features

The normal epidermis layer consisted of three to four rows of basal cells, squamous cells, granulosa cells, and non-nucleated keratinocytes in the stratum corneum. The normal dermis exhibited minimal presence of inflammatory cells and blood vessels in the papillary dermis (**Figure 3** A). After the

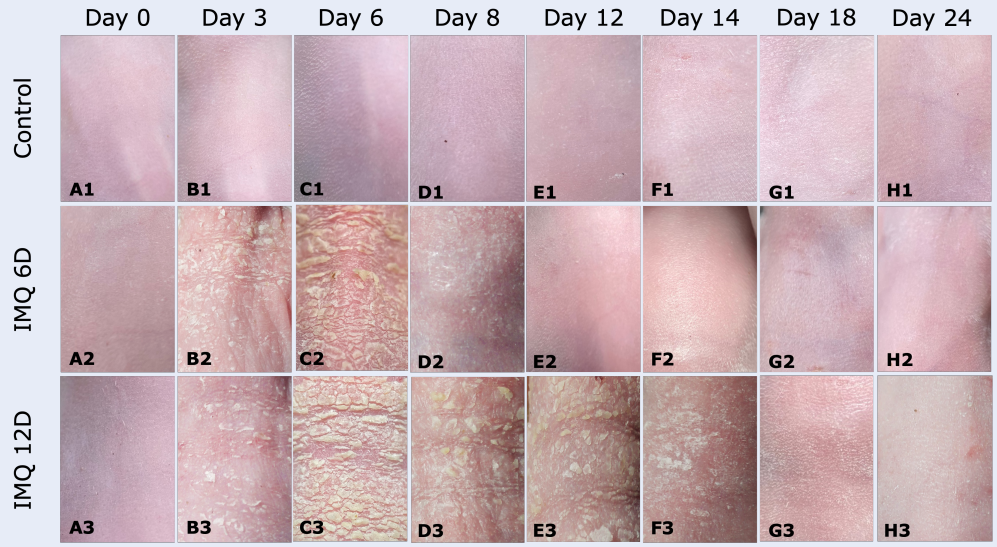

Figure 1: **Imiquimod-induced psoriasis-like clinical features**. Representative of skin on days 0,3, 6, 8, 12, 14, 18, and 24 at: normal mice (A1-H1); IMQ topical application mice for 6 days (A2-H2) and 12 days (A3-H3). **Abbreviations**: **IMQ**: imiquimod; **D**: day

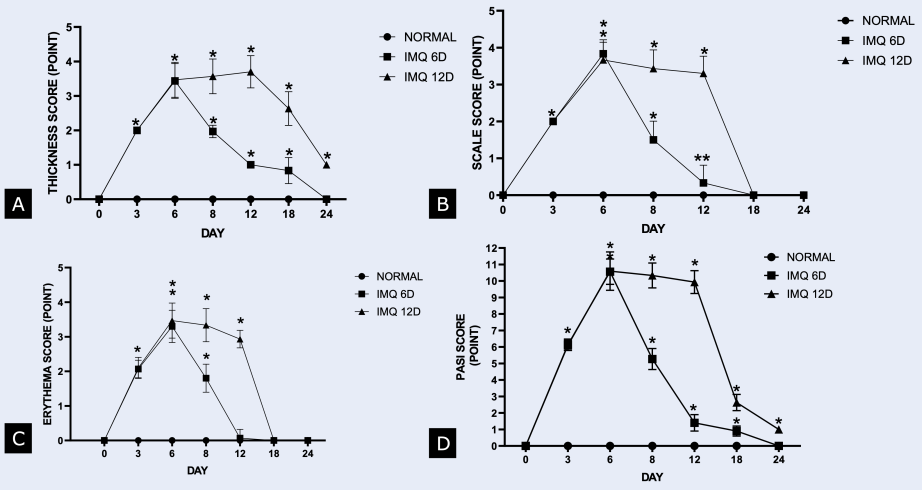

Figure 2: **Severity scores of psoriasis-like lesions induced by IMQ over time**. The score of thickness (**A**), scaling (**B**), erythema (**C**) and the combined cumulative (**D**) of the dorsal skin from day 0 to 24 at normal mice skin and mice psoriasis-like lesions skin induced by IMQ over time from 6 days IMQ application group (IMQ 6D), and 12 days IMQ application group (IMQ 12D) (n = 30). The statistical difference between the normal group and the other two groups of mice was calculated using a Student's t-test, * $p < 0.0001$, ** $p < 0.001$. **Abbreviations**: **IMQ**: imiquimod

Table 1: Mouse primer sequences for real-time quantitative polymerase chain reaction

Target gene	Sequenses (5'-3')	Product lenght (bp)	Accession number
Gapdh	F: GCA TCT TCT TGT GCA GTG CC; R: TAC GGC CAA ATC CGT TCA CA	74	NM_008084.4
IL-17A	F: CCC CTT CAC TTT CAG GGT CG; R: GGG GGT TTC TTA GGG GTC AG	116	NM_010552.3
IL-23	F: TGG AGC AAC TTC ACA CCT CC; R: GGC AGC TAT GGC CAA AAA GG	170	NM_031252.2

F: forward; **R**: reverse; **IL**: Interleukin

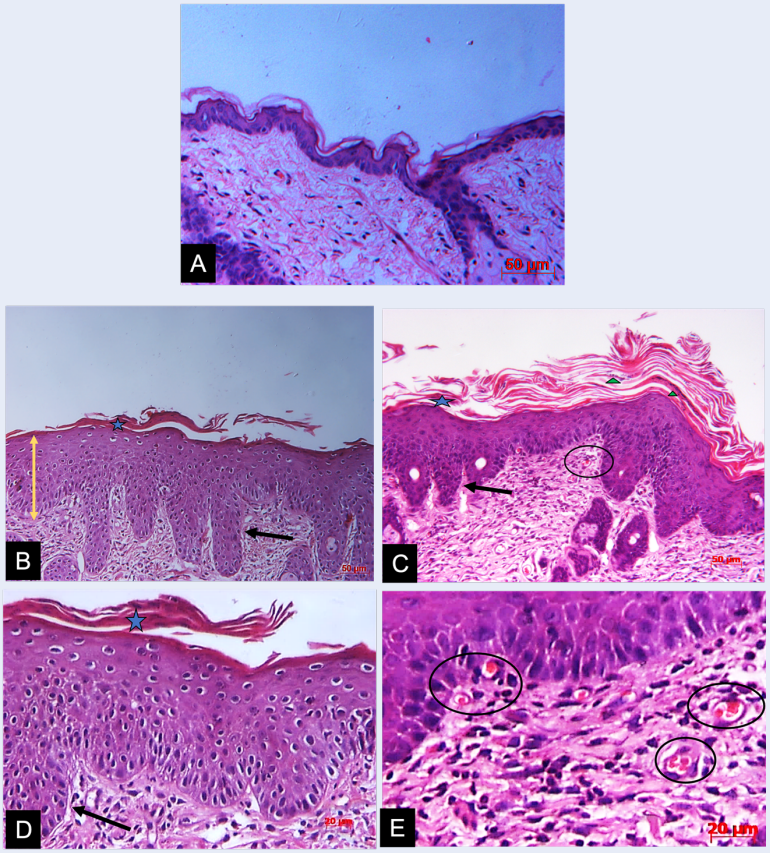

Figure 3: **Histopathological feature of skin lesion on day 6**. H&E staining of (**A**) normal mouse skin and psoriasis-like skin induced by IMQ on day 6 (**B-E**) reveals several characteristic features: epidermal hyperplasia (double-headed yellow arrows), parakeratosis with nuclei in the stratum corneum (indicated by stars), Munro's microabscesses (indicated by triangles), acanthosis with elongated rete ridges (indicated by arrows), and dilated, tortuous vasculature (indicated by circles). For each specimen, three sections were selected, and for each section, three photos were taken. Scalebar: 50 μm. **Abbreviations**: **IMQ**: imiquimod; **H&E**: Hematoxylin-Eosin

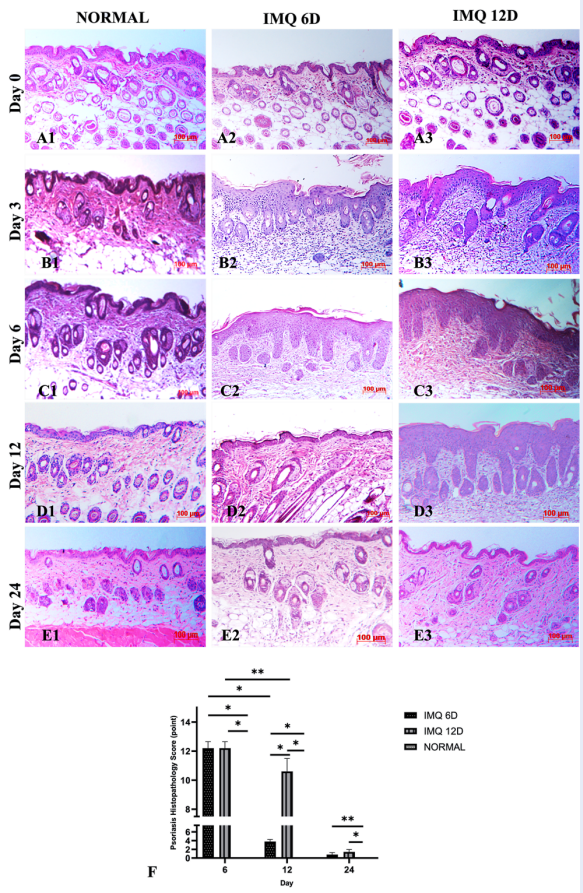

Figure 4: **Imiquimod - induced psoriasis-like histopathological features.** Hematoxylin-Eosin staining of normal mice skin (**A1-E1**) and of psoriasis-like lesions induced by IMQ over time from 6 days IMQ application group (**A2-E2**), and 12 days IMQ application group (**A3-E3**) (n = 5). PHS was calculated at day 6 and day 12 of three group (**F**): Normal group, 6 days IMQ application group (IMQ 6D), 12 days IMQ application group (IMQ 12D) (n = 5). *P < 0.001, **P < 0.01. For each specimen, three sections were selected, and for each section, three photos were taken. Scalebar: 100 μm **Abbreviations**: **IMQ**: imiquimod; **PHS**: Psoriasis Histopathology Score

application of IMQ, distinct changes characteristic of psoriasis became evident in both the epidermis and dermis of the mouse dorsal skin. In the epidermis, psoriasis-like features were observable, including hyperkeratosis and hyperplasia with keratinocytes proliferating to form a thickened layer of keratin (**Figure 3** C). Notably, corneocytes retained nuclei within the cells, a condition referred to as parakeratosis (indicated by stars, **Figure 3** B-D). Additionally, neutrophils accumulated within the corneum layer, giving rise to Munro's microabscesses (highlighted by triangles, **Figure 3** C). The epidermis displayed thickening with multiple layers of cells (denoted by double-headed yellow arrows, **Figure 3** B). Furthermore, the granular layer was absent, and regular acanthosis with elongated rete ridges extended into the dermis (indicated by arrows, **Figure 3** B-C). In the dermis, there was a notable increase in the infiltration of inflammatory cells, predominantly lymphocytes, and evidence of small blood vessel proliferation at the apex of the dermal papilla (indicated by circles, **Figure 3** C, E). Throughout the experiment, skin samples from normal batches of mice maintained a consistent histopathological appearance (**Figure 4** A1-A3, B1-E1), with a PHS of 0 when evaluated on both days 6 and 12 (**Figure 4** F). In contrast, both the IMQ 6D and IMQ 12D groups exhibited characteristics of psoriatic epidermal hyperplasia from day 3 (**Figure 4** B2-3), which became most pronounced by day 6 (**Figure 4** C2-3). The PHS reached its peak value of 12.2 $\pm$ 0.45 on day 6 after IMQ application, significantly higher compared to the normal group (**Figure 4** F). Continuous IMQ application for 12 days led to a reduction in psoriatic epidermal hyperplasia (**Figure 4** D3), with

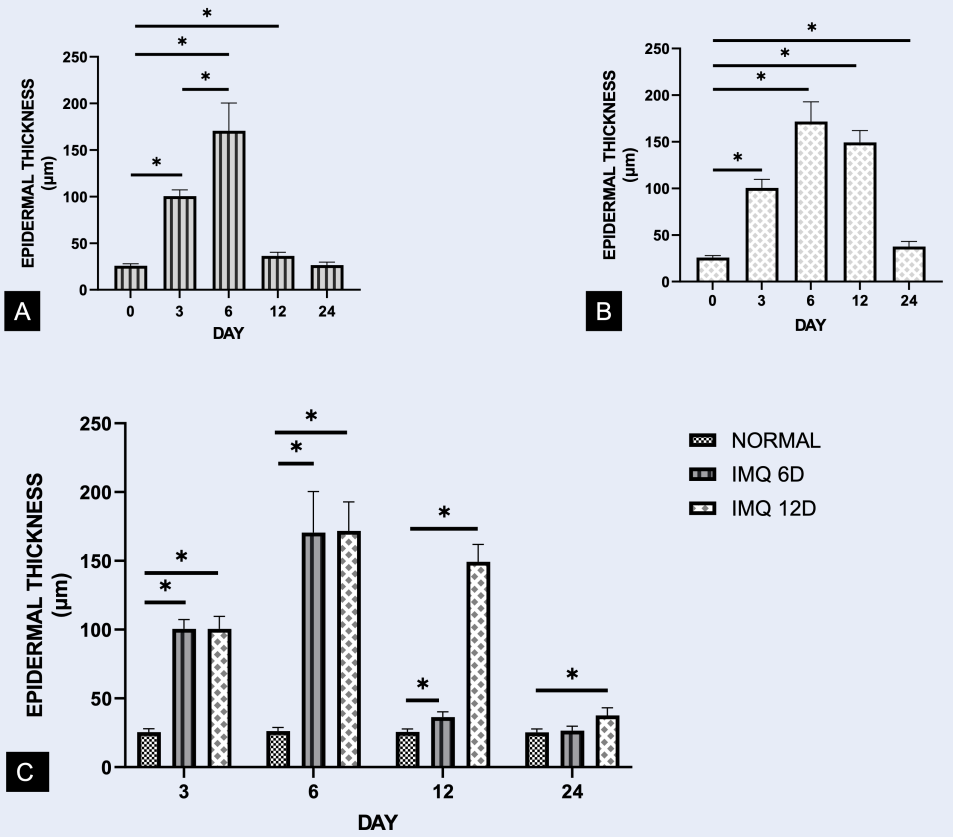

Figure 5: **Epidermal thickness of psoriasis-like lesions**. The epidermal thickness of psoriasis-like lesions induced by IMQ increased from day 0 to day 6 and gradually decreased from day 6 to day 24 in the 6-day IMQ application group (**A**). In the 12-day IMQ application group, the epidermal thickness increased from day 0 to day 12 and then decreased from day 12 to day 24 (**B**). From day 3 to day 12, the epidermal thickness of the normal mouse group (Normal) was lower than that of the IMQ-treated groups for 6 days (IMQ 6D) and 12 days (IMQ 12D). On day 24, the epidermal thickness in the 6-day IMQ application group returned to normal, while the thickness in the 12-day IMQ application group remained higher than that of the normal group (**C**). * p<0.0001. **Abbreviations**: **IMQ**: imiquimod.

the granulosum stratum still present in some mice. The frequency of Munro's microabscesses, elongation of the rete ridges, and small blood vessel proliferation at the apex of the dermal papilla decreased. The score of psoriasis histopathological features in this group was 10.6 ± 0.89 (**Figure 4** F), significantly different from day 6 ($p < 0.01$).

Following discontinuation of IMQ application on day 6, psoriasis histopathological features gradually diminished. By day 12, there was reduced parakeratosis, absence of regular elongation of the rete ridges, appearance of the granular layer, and the absence of Munro's microabscess. In the dermis, moderate inflammatory cell infiltration persisted with lymphocytes predominating, although the proliferation of small blood vessels at the papillary dermis decreased (**Figure 4** D2, E2). The PHS decreased to 3.8 ± 0.45, significantly lower than that of the IMQ 12D group (**Figure 4** F) ($p < 0.001$). Psoriasis histopathological features gradually diminished after discontinuing IMQ from day 12. On day 24, skin samples from both the IMQ 6D and IMQ 12D groups exhibited minimal histopathological manifestations of psoriasis compared to day 6. The infiltration of inflammatory cells was significantly reduced (**Figure 4** E2, E3). The PHS scores were 0.8 ± 0.45, not significantly different compared to the normal group, and 1.4 ± 0.55, significantly different compared to the normal group, respectively (**Figure 4** F) ($p < 0.05$). The average epidermal thickness of normal mice, as observed in histological samples stained with hematoxylin and eosin, was 25.86 ± 2.08 μm. This thickness remained relatively

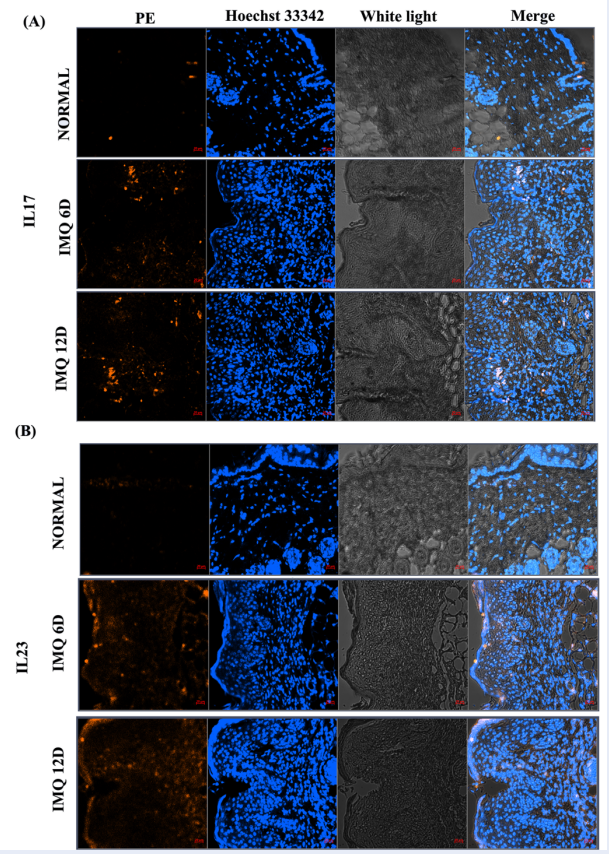

Figure 6: **IL-17A and IL-23p19 infiltration on skin lesion at day 6**. The expression levels of IL-17A (**A**) and IL-23p19 (**B**) in the skin of mice were evaluated on the day 6 in normal mice, mice exposed to IMQ for 6 days (IMQ 6D), and mice exposed to IMQ for 12 days (IMQ 12D). For each specimen, three sections were selected, and for each section, three photos were taken. Scalebar: 20 μm. **Abbreviations**: **IMQ**: imiquimod; **IL**: interleukin.

consistent throughout the study period, showing no significant fluctuation (**Figure 5** C). Upon the application of IMQ, a notable increase in epidermal thickness was observed. The upper layer thickness in both the IMQ 6D and IMQ 12D group was similar, with values of 170.5 $\pm$ 29.84 μm and 171.8 $\pm$ 21.03 μm, respectively (**Figure 5**). This increase was statistically significant compared to the epidermal thickness observed in mice of the normal group ($p < 0.001$) (**Figure 5** C). The thickness slightly decreased to 149.3 $\pm$ 12.66 μm with continuous IMQ application until the 12^{th} day (**Figure 5**). After discontinuation of IMQ on day 6, the epidermal thickness decreased to 36.45 $\pm$ 3.76 μm on day 12 and nearly returned to the normal value by day 24 (**Figure 5** A). However, in the IMQ 12D group, despite a decrease in epidermal thickness from day 12, a notable thickness persisted on day 24, with a value of 37.60 $\pm$ 5.48 μm (**Figure 5** B).

Expressions of IL-23/IL-17A in IMQ-treated Skin According to Time

Concerning the expression of IL-17A and IL-23 in the immunofluorescence-stained samples, normal immunofluorescence staining samples revealed minimal or negligible infiltration of IL-17A and IL-23 in the epidermis and dermis on days 6 and 12 of the experiment (**Figures 6 and 7**). On day 6 after IMQ application, both the IMQ 6D and IMQ 12D groups exhibited evident concentrations of IL-17A (**Figure 6** A) and IL-23 (**Figure 6** B) in the dermis, with smaller amounts distributed in the epidermis. Continuing to day 12, in the IMQ 12D group, IL-17A infiltration persisted in both the dermis and epidermis, albeit at reduced levels. Upon cessation of IMQ application from day 6, a similar reduction in IL-17A infiltration was observed, with a more pronounced reduction in epidermal infiltration compared to the group with continued IMQ application (**Figure 7** A).

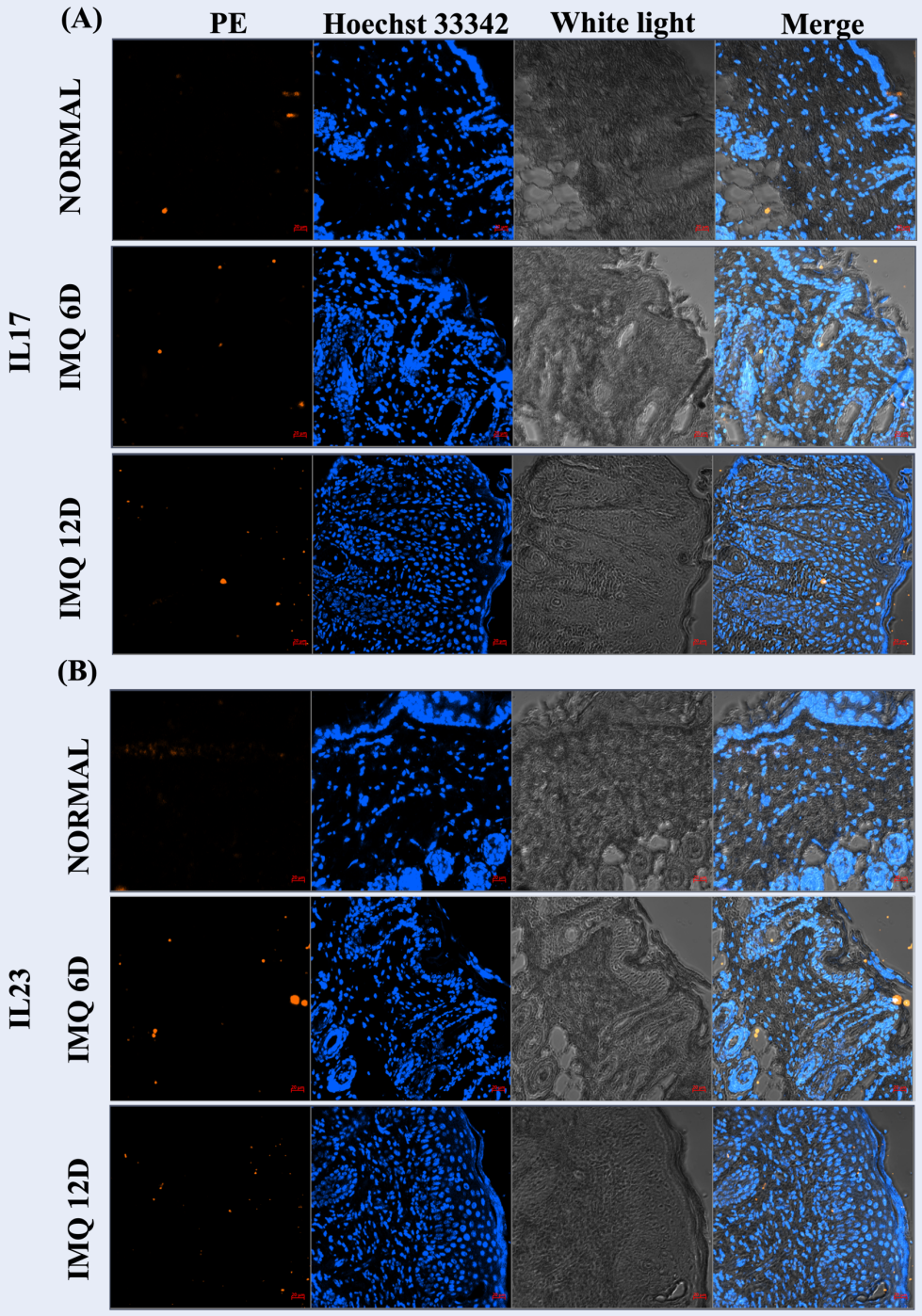

Figure 7: **IL-17A and IL-23p19 infiltration on skin lesion at day 12**. The expression levels of IL-17A (**A**) and IL-23p19 (**B**) in the skin of mice were evaluated on day 12 in normal mice, mice exposed to IMQ for 6 days (IMQ 6D), and mice exposed to IMQ for 12 days (IMQ 12D). For each specimen, three sections were selected, and for each section, three photos were taken. Scalebar: 20 μm. **Abbreviations**: **IMQ**: imiquimod; **IL**: interleukin

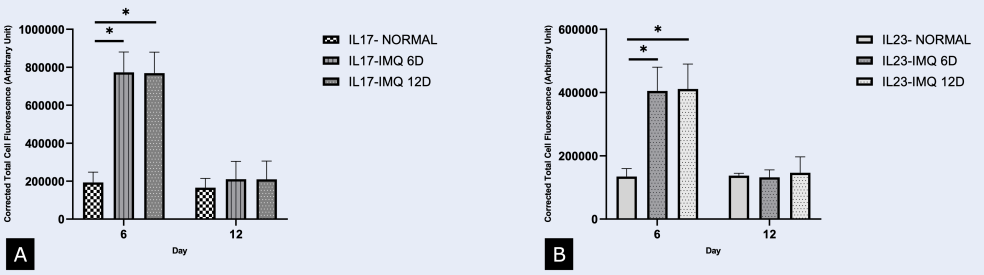

Figure 8: **The expression levels of IL-17A (A) and IL-23 (B) in mice skin lesions over time were calculated using Corrected Total Cell Fluorescence**. On day 6, the fluorescence intensity of IL-17A and IL-23 increased significantly in the immunohistochemical stained samples from mice treated with IMQ for 6 and 12 days. On day 12, the fluorescence intensity of both IMQ-treated groups decreased to levels comparable to the normal mouse group; (n = 5). *P < 0.001. **Abbreviations**: **IMQ**: imiquimod; **IL**: interleukin

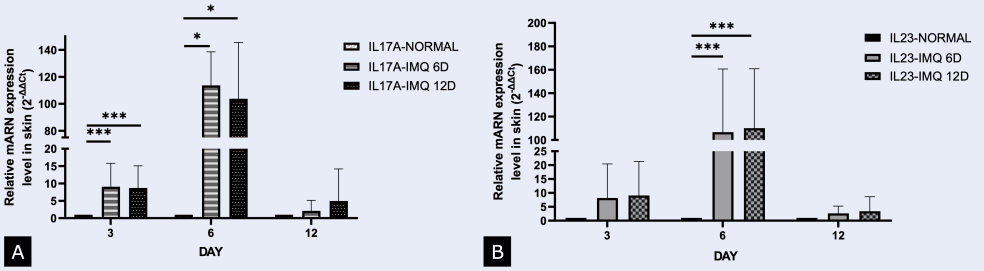

Figure 9: **mARN expression of IL-17A and IL-23 in the skin lesion over time**. mARN expression of IL-17A (**A**) and IL-23 (**B**) targeted genes in mice skin lesions over time
(n = 5). *P < 0.0001 compared to normal group. The mARN expression levels of IL-17A and IL-23 increased from day 3 to day 6 with IMQ application. This expression returned to normal on day 12, regardless of continued IMQ application or not. **Abbreviations**: **mARN**: messenger ribonucleic acid; **IMQ**: imiquimod; **IL**: interleukin

The expression pattern of IL-23 mirrored that of IL-17A throughout the experiment. On day 12, IL-23 expression significantly decreased in both the epidermis and dermis, regardless of whether IMQ application continued. However, in the group that discontinued IMQ application from day 6, the reduction in IL-23 infiltration was more pronounced compared to the group with continued IMQ application for 12 days (**Figure 7** B). The expression levels of IL-17A and IL-23 in skin lesions of mice over time, assessed using Corrected Total Cell Fluorescence (CTCF), were consistent with the patterns observed in the immunofluorescence staining images. Following IMQ application, the CTCF values for IL-17A (**Figure 8** A) and IL-23 (**Figure 8**B) increased significantly, by 3 and 2 times respectively, compared to the normal group on day 6, followed by a notable decrease on day 12, approaching normal values. Meanwhile, the CTCF values for both IL-17A and IL-23 in normal skin of the normal group remained constant throughout the experimental period (**Figure 8**). Based on the protein expression of IL-17A and IL-23 in immunohistochemically stained samples, we assessed the gene expression levels of these proteins at different time points. On the third day following IMQ application, the mRNA expression of IL-17A and IL-23 increased significantly, by 9.08 $\pm$ 6.71 and 8.17 $\pm$ 12.24 times, respectively, compared to the normal group. This elevation was statistically significant for IL-17A. The expression levels continued to escalate, reaching their peak on day 6 of IMQ application, with the mRNA expression of IL-17A and IL-23 soaring by 113.7 $\pm$ 24.93 and 106.7 $\pm$ 53.88 times, respectively, significantly higher than those of the normal group. Subsequently, expression gradually declined. However, on day 12, after 12 days of IMQ application, IL-17A and IL-23 expression levels were 4.95 $\pm$ 9.21 and 3.4 $\pm$ 5.23 times higher than those of the normal group, respectively. Although these values exceeded the corresponding expression levels in the IMQ 6D group, the differences in expres-

sion between the two groups were not statistically significant (**Figure 9**).

DISCUSSION

In this study, we demonstrated that the psoriasis-like model in mice, induced by 6 or 12 days of IMQ application, exhibited characteristic features similar to psoriasis. These included morphological traits such as erythematous plaques, white scales, and thickened skin, alongside histopathological findings like acanthosis, parakeratosis, Munro microabscesses, neoangiogenesis, and inflammatory cell infiltration. While these manifestations persisted throughout the daily application period until day 12, they became less pronounced compared to day 6. Following the cessation of IMQ application, both skin manifestations and histopathological abnormalities gradually resolved by day 24. Furthermore, IL-17A and IL-23 exhibited increased expression during the application period, which declined upon discontinuation, corresponding to a reduction in clinical manifestations.

Several models have been developed to study psoriasis treatment, encompassing direct induction, spontaneous induction, genetic engineering, and xenotransplantation methods[5,6]. The xenograft model, which involves grafting psoriatic skin tissue onto immunocompromised mice, closely simulates psoriasis-like skin conditions but is complex, costly, and demands skilled personnel. On the other hand, the direct induction model, achieved by applying imiquimod (IMQ) to mouse skin or injecting interleukin-23 (IL-23) subcutaneously, is more accessible and cost-effective. Among these, the IMQ-induced model is particularly noteworthy, demonstrating clinical, histopathological, and immunological similarities to psoriasis, thereby making it extensively utilized[16].

Van der Fits *et al.* initially reported the induction of psoriasis-like lesions through the topical application of 5% imiquimod (IMQ) on mouse skin for a continuous period of six days[7]. They emphasized the critical role of the IL-17/IL-23 axis in the pathogenesis of these lesions. Following this seminal study, various researchers have utilized this model to assess psoriasis treatments, typically adhering to the six-day application protocol for acute lesion induction[10–12]. However, to evaluate long-term treatment efficacy or to establish a chronic model, extended periods of IMQ application are deemed necessary. This introduces a significant query: does continuous IMQ application beyond six days preserve the integrity of the model or result in a chronic psoriasis-like condition? Our study is designed to examine the characteristics of psoriasis in mouse models under both short-term and long-term IMQ exposure, representing the first initiative to systematically investigate this dimension of the IMQ-induced psoriasis-like model.

In this study, we conducted a pilot investigation and noted that by day 6, mice displayed prominent clinical signs of psoriasis, characterized by erythematous scaly plaques and skin thickening. Subsequently, upon the discontinuation of IMQ application, by day 12, nearly all psoriasis-like lesions on the mice's skin had resolved. However, certain treatments may require a longer maintenance phase than 6 days to demonstrate therapeutic efficacy and may necessitate repeated dosing. Therefore, we chose to conduct an additional 6-day modeling cycle until day 12 to attain optimal psoriasis-like lesions for treatments necessitating prolonged intervention.

Our research findings indicate that a psoriasis-like condition in mice, following 6 or 12 days of IMQ application, demonstrates characteristics similar to psoriasis. These include erythematous plaques, white scales, and thickened skin, aligning with previous studies that utilized an acute direct induction psoriasis model[17–19]. Symptoms begin to manifest on day 3 and peak by day 6, suggesting these time points as optimal for initiating treatment trials aimed at mitigating psoriasis-like lesions. The severity of symptoms peaks on day 6, making it an ideal time for assessing the effectiveness of treatments. However, starting treatment at this peak stage may not effectively alleviate symptom severity. Treatment initiation on day 3, when lesions are prominent yet moderate, could be more beneficial. Evaluating treatment efficacy on day 6 allows for an assessment of improvement in psoriasis-like features due to intervention. The gradual reduction in clinical manifestations after ceasing IMQ application implies that evaluations of treatment efficacy conducted solely after day 6 might not capture significant differences. Furthermore, the 6-day IMQ-induced psoriasis-like model may not sufficiently evaluate the long-term effects of therapeutic interventions.

To evaluate histopathological alterations, we scrutinized changes in the epidermis, dermis, and hypodermis of mouse skin following treatment with IMQ across a designated timeframe. Observable clinical symptoms among various mouse groups emerged on days 3, 6, 12, and 24, leading us to perform skin biopsies and stain tissue samples from these intervals. Echoing prior studies, our results revealed that starting from day 3, the dorsal skin of mice displayed distinct histopathological characteristics akin to those seen in human psoriasis lesions[7]. Noted changes included regular acanthosis with rete ridge elongation,

hyperkeratosis accompanied by parakeratosis, a reduced granular layer, neutrophil infiltrates within the stratum corneum forming Munro's microabscesses, predominantly lymphocytic infiltrate in the upper and middle portions of the dermis, dilated and tortuous vessels in the dermal papilla. These features aligned with the histopathological benchmarks for a psoriasis-like model outlined by Nestle and Nickoloff, encompassing: 1) epidermal modifications marked by increased keratinocyte proliferation and irregular differentiation; 2) the development of elongated and symmetrical rete ridges; 3) the infiltration of inflammatory cells; and 4) vascular alterations[7]. Such psoriasis-analogous signs persisted until day 12 post-continuous IMQ application, although they were less pronounced compared to day 6. By day 12, the histopathological characteristics had diverged from the specificity seen on day 6, including diminished epidermal thickness and a deviation from the second criterion as per Nestle and Nickoloff. Consequently, the sixth day represented an optimal juncture for assessing treatment efficacy.

Our research found that after stopping IMQ treatment, skin symptoms and histopathological changes steadily diminished and vanished by day 24. In a short-term IMQ treatment scenario lasting 6 days, we observed a notable decrease in erythema and scaling, paralleled by significant reductions in pathological anomalies by day 12, leading to the complete resolution by day 24. The normalization of skin and epidermal thickness by day 24, 18 days post-IMQ discontinuation, signifies recovery. For the extended IMQ treatment of 12 days, reductions in erythema, scaling, and pathological changes were also significant after ceasing IMQ for 6 days. Yet, the thickness of both skin and epidermis within the pathological sites persisted until day 24. This indicates the possibility of preserving both the clinical appearance and some pathological aspects of psoriasis in a mouse model by prolonging IMQ treatment, but they are less marked than at day 6. These effects significantly diminish upon halting IMQ treatment for 6 days.

The pathogenesis of psoriasis remains partially understood, yet the IL-23/IL-17 axis has been identified as a pivotal factor. This axis's significance is also highlighted in the IMQ-induced mouse model for psoriasis, where it is closely linked with inflammatory skin reactions, as evidenced in studies[7]. IMQ, acting as a TLR7 agonist and employed in treating genital warts, has been shown to provoke psoriasis-like lesions through its effect on TLR7. The mechanism involves IMQ's interaction with TLR7 and dendritic cells, leading to an upsurge in interleukins tied to the Th17 pathway, such as IL-17 and IL-23. This interaction results in modifications to the skin's epidermis and dermis[8].

The alterations in histopathological features noted in our study are attributed to cytokine expression. To further understand these changes, we assessed the expression of IL-17A and IL-23 at the lesion sites. Following 3 days of IMQ treatment, a marked increase in IL-17A expression was observed at the sites of lesions. By day 6, the expressions of both IL-17A and IL-23 were significantly more pronounced. These findings are in line with those of previous studies[20]. Moreover, mice deficient in IL-23p19/IL-17RA did not develop psoriasis-like lesions[9], indicating a positive correlation between IL-17A expression and disease progression[21]. Additionally, the employment of IL-17A antagonists or agents reducing IL-17A production has been documented to lessen disease severity in this model[20,22], underscoring the critical role of the IL-23/IL-17 axis in the context of short-term IMQ application and its potential for psoriasis treatment trials targeting this pathway.

Our research also uncovered that IL-23 was abundantly expressed in both the dermis and epidermis, whereas IL-17A showed a significant presence in the dermis with moderate levels in the epidermis. This pattern reflects the progression of the inflammatory process leading to psoriasis-like lesions, with IL-23 serving as the upstream interleukin. The IMQ-induced production of IL-23 from dendritic cells through TLR7 activation in the dermis[8] further promotes Th17 differentiation and IL-17 release into both skin layers. Notably, after ceasing the modeling agent, a more rapid decline in IL-17 levels was observed in the epidermis compared to the dermis in our study.

After stopping IMQ treatment on day 6, the expressions of IL-17A and IL-23 quickly diminished. This trend aligns with Li et al.'s recent findings. However, their investigation did not extend beyond day 12, thereby not evaluating the model's capability for chronic simulation[20].

In conclusion, the 6-day IMQ model demonstrated an increase in IL-17A and IL-23 levels during treatment and a decrease following cessation, correlating with reduced clinical manifestations. These results suggest that the efficacy of IL-17A/IL-23 antagonists in this model can be assessed by monitoring changes in these interleukins alongside clinical and histological signs.

Despite continuous IMQ application from day 6 to 12, the expressions of IL-17A and IL-23 decreased by day 12, indicating that these interleukins might not

solely account for the persistent clinical and histological signs upon extending IMQ application duration. This model suggests additional mechanisms, such as inflammasome activation via the NALP3 pathway, adenosine receptor signaling interference, or the biological activity of the vehicle[8,16], may contribute to the formation of psoriasis-like lesions, even with a significant decrease in IL-17A and IL-23 expression with continued drug administration. Thus, treatments targeting pathways other than the IL-17A/IL-23 may find utility in this model, with clinical and histological response monitoring as key factors. Further exploration into these mechanisms is essential, while the short-term IMQ application model remains recommended for evaluating psoriasis treatments targeting the IL-17A/IL-23 pathway.

Overall, this study delved deeper into the IMQ-induced psoriasis model, offering comprehensive insights into the time-dependent effects of imiquimod application durations on psoriasis-like lesions and cytokine expression in mice. This model, reliant on the IL-23/IL-17 pathway, effectively replicates key aspects of psoriasis. However, careful consideration of the duration of IMQ application is necessary for accurate model assessment and understanding. Moreover, it enhances our comprehension of the role of IL-23/IL-17 in psoriasis pathogenesis. Additionally, due to its convenient modeling method and clear relation to IL-23/IL-17 signaling, this model proves to be a valuable tool for preclinical screening of IL-23/IL-17 antagonists, thus advancing psoriasis treatment evolution.

Nevertheless, our study faces several limitations. Firstly, while the IMQ model primarily induces a Th17-driven response, akin to psoriasis, it may not encapsulate all aspects of human psoriasis. Thus, it is most useful in studying pathogenic mechanisms related to IL-17/IL-23 and treatments through IL-17/IL-23 inhibition. Secondly, while our study focuses on 6-day and 12-day IMQ application durations, longer durations or varied treatment times could provide further insights into chronic aspects of psoriasis and sustainability of cytokine expression changes. Observations beyond day 24 could offer crucial information on the long-term effects of IMQ treatment cessation, relevant for understanding disease relapse or recovery. Additionally, discrepancies between cytokine levels and clinical expression necessitate further research into pathogenic mechanisms underlying psoriasis-like lesions.

Thirdly, the use of *Balb/c* mice, while suitable for a 6-day application period, may not fully represent varied immune responses seen in different strains. Pilot studies showed no gender differences in clinical and immune phenotypes; hence, both genders were included to mimic psoriasis in humans. Fourthly, while our sample sizes were sufficient to enhance statistical power and provide definitive insights, larger studies are warranted to confirm our results.

Finally, while IL-17 and IL-23 are critical, expanding analysis to include other cytokines and signaling molecules could offer a more comprehensive understanding of psoriasis. We hope these findings promote the use of the IMQ-induced psoriasis mouse model in future research, aiding in evaluating the efficacy and safety of potential psoriasis treatments.

CONCLUSIONS

Our research clearly illustrates that a six-day regimen of topical Imiquimod application effectively induces psoriasis-like lesions in mice, thereby closely simulating human psoriasis via the IL-23/IL-17 pathway. This emphasizes its potential as a model for assessing therapies targeted at the IL-23/IL-17 axis. We observed that extending the application of Imiquimod beyond this period does not improve the model's accuracy, indicating that the six-day protocol is adequate for psoriasis studies. The sustained presence of psoriatic characteristics after prolonged Imiquimod application warrants further exploration into the persistent aspects of this disease model. Our results underline the importance of the six-day Imiquimod model in psoriasis research, setting the stage for future investigations to evaluate treatment options, understand the molecular pathways underpinning cytokine dynamics, and examine the mechanisms behind the extended manifestation of the disease. This research thereby enhances the development of novel therapeutic approaches.

ABBREVIATIONS

CTCF - Corrected Total Cell Fluorescence; **DCs** - Dendritic Cells; **GAPDH** - Glyceraldehyde 3-Phosphate Dehydrogenase; **H&E** - Hematoxylin and Eosin; **HCMUS** - Ho Chi Minh City University of Science; **IL-17** - Interleukin-17; **IL-17A** - Interleukin-17A; **IL-23** - Interleukin-23; **IMQ** – Imiquimod; **mARN** - Messenger Ribonucleic Acid (Typically referred to as mRNA, the provided abbreviation might be a typographical error); **PASI** - Psoriasis Area and Severity Index; **PCR** - Polymerase Chain Reaction; **PHS** - Psoriasis Histopathology Score; **RT-qPCR** - Real-Time Quantitative Polymerase Chain Reaction; **TLR** - Toll-Like Receptor; **TLR7** - Toll-Like Receptor 7

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AUTHOR'S CONTRIBUTIONS

Trung The Van, Ngoc Bich Vu, Thanh Thai-Van Le, Phuong Thi-Thuy Tran: Critical literature review; study conception and planning. Phuong Thi-Thuy Tran, Loc Tan Le: Data collection. Phuong Thi-Thuy Tran, Ngoc Bich Vu: Statistical analysis, writing- original draft preparation editing. Phuong Thi-Thuy Tran; Ngoc Bich Vu, Trung The Van: Data analysis and interpretation, writing – review. All authors read and approved the final manuscript.

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AVAILABILITY OF DATA AND MATERIALS

Processed data and materials used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All animal procedures were approved by the Animal Ethics Committee of the Stem Cell Institute, University of Science, Vietnam National University Ho Chi Minh City, Viet Nam (Ref number: 220102/SCI-ACE).

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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