IL-36γ promotes psoriasis-like features in keratinocytes in an imiquimod-induced murine model of psoriasis

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Abstract

Introduction: Psoriasis is a recurrent, chronic inflammatory skin disease with a complex pathogenesis. The disease imposes a heavy burden on patients. Interleukin (IL)-36γ belongs to the IL-36 family and is predominantly expressed by epithelial cells. IL-36γ is upregulated in psoriasis lesions. However, the effects of IL-36γ in keratinocytes remain unclear.

Material and methods: Eighteen IL-36γ-deficient mice were divided into three groups: the Vaseline group, the imiquimod (IMQ) group, and the IMQ/IL-36γ group. Vaseline or IMQ was administered for 6 consecutive days. The severity of psoriasis-like lesions was evaluated using a modified Psoriasis Area and Severity Index (PASI) scoring system. Production of cytokines and expression of differentiation markers were assessed by immunohistochemistry.

Results: IMQ-induced psoriasis lesions were significantly more severe in IMQ/IL-36γ-treated mice compared with Vaseline-treated and IMQ-treated mice, as shown by an exacerbated inflammatory phenotype, increased numbers of blood vessels, increased infiltration of cells, and increased epidermal thickness. Expression of loricrin and keratin 5 in skin lesions was decreased following treatment with IL-36γ. Levels of IL-17A, interferon-γ, β-catenin and Dickkopf-related protein 1 were elevated in keratinocytes within psoriatic lesions following IL-36γ stimulation.

Conclusions: Together, these data showed that IL-36γ contributes to abnormal keratinocyte proliferation and keratinocyte-related proinflammatory cytokines, and suggest that IL-36γ may play an important role in the pathogenesis of psoriasis.

Key words: IL-36γ, psoriasis, Psoriasis Area and Severity Index.

Introduction

Psoriasis is a chronic, immune-mediated inflammatory skin disorder that is characterized by well-demarcated, scaly, erythematous plaques. The mechanisms underlying psoriasis are not fully understood but the condition is generally attributed to dysfunction of the epithelial and immune systems triggered by stimuli such as infections, medications, and trauma. Psoriasis vulgaris is the most prevalent type of psoriasis, accounting for approximately 90% of all cases [1]. Histologically, psoriatic skin lesions are typically characterized by uncontrolled keratinocyte proliferation, aberrant differentiation of keratinocytes, dilated and

hyperplastic blood vessels, and inflammatory infiltration of leukocytes (including dendritic cells, macrophages, T cells, and neutrophils) into the dermis [2]. The interactions among keratinocytes, cytokines, and immune cells play crucial roles in the formation of psoriatic lesion [2, 3].

Interleukin (IL)-36 cytokines, including IL-36α, IL-36β and IL-36γ, belong to the IL-1 family of cytokines. The IL-36 receptor antagonist (IL-36Ra) is an antagonist of IL-36 function [4]. IL-36 cytokines and IL-36Ra are abundant in the skin and participate in epidermal cornification. IL-36 is produced by various cell types including keratinocytes, epithelial cells, tissue resident macrophages, monocytes, and dendritic cells. Several studies have reported that expression of IL-36γ was upregulated in the lesions of psoriasis patients and was associated with disease activity [5, 6]. However, the effect of IL-36γ on keratinocytes remains unclear. In this study, we investigated the effects of IL-36γ on keratinocytes in an imiquimod (IMQ)-induced murine model of psoriasis.

Material and methods

Animal experiments

All experimental mice were of the C57BL/6J background. IL-36γ-deficient mice were generated by Shanghai Model Organisms (Shanghai, China). All mice were maintained under specific pathogen-free conditions and provided with a standard laboratory diet and water. The mice were randomly divided into three groups (n = 6; three males and three females per group): the control group, the IMQ group, and the IMQ/IL-36γ group. Mice received a daily topical dose of 62.5 mg 5% imiquimod cream (Aldara, 3M Pharmaceuticals, UK) or Vaseline on the shaved dorsal skin for 6 consecutive days. The mice in the IMQ/IL-36γ group were treated with 2 μg of IL-36γ (R&D Systems, Minneapolis, MN, USA) by subcutaneous injection on days 1, 3, and 5. The severity of psoriasis-like lesions was evaluated using a modified Psoriasis Area and Severity Index (PASI) scoring system. The modified system was adapted from the clinical PASI system, which includes three criteria: erythema, scaling, and thickness. Two parameters were scored independently on a scale from 0 to 4 according to external physical appearance: 0 = none, 1 = slight, 2 = moderate, 3 = marked, and 4 = severe.

Immunohistochemistry

Psoriatic skin samples from three groups were fixed in 10% formalin and embedded in paraffin for hematoxylin and eosin (H&E) staining and histopathology. The sections were scanned using the Hamamatsu Digital Pathology System and epidermal thickness was measured using NDP.view software (Hamamatsu Photonics, Hamamatsu, Japan). For immunohistochemistry analysis, formalin-fixed and paraffin-embedded skin sections were incubated with primary antibodies against IL-17 (Abcam, Cambridge, MA, USA), IL-6 (Abcam), interferon (IFN)-γ (Abcam), keratin 5 (Abcam), keratin 17 (Abcam), β-catenin (Abcam), and loricrin (Proteintech, Rosemont, IL, USA). Subsequently, the sections were incubated with horseradish peroxidase-conjugated secondary antibodies (Proteintech). Then, 3,3′-diaminobenzidine (Proteintech) was used as a substrate. The sections were scanned using the Hamamatsu Digital Pathology System.

The H-score method was used to quantify staining intensity from immunohistochemistry. The range of the H-score was 0–300 based on the percentage of cells at different staining intensities. The relative intensity of specific staining was defined as 0 (no staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining). The final score was the sum of the relative staining intensity (0–3) multiplied by the percentage of positive cells. The H-score analysis was carried out using microscopy by two experienced pathologists in a double-blind fashion.

Statistical analysis

Data were presented as means ± standard deviations. Statistical analysis was performed using SPSS 21.0 (IBM Corp., Armonk, NY, USA). Two-tailed tests were used in all analyses and values of $p < 0.05$ were considered statistically significant. Differences in H-score data and PASI scores were assessed using the Mann-Whitney U test. Differences between continuous variables were assessed using an independent-sample t-test. Figures were produced using GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA).

Results

IL-36γ aggravates the psoriasiform phenotype of IMQ-treated mice

To investigate the effects of IL-36γ on IMQ-induced psoriasis in mice, IL-36γ was subcutaneously injected on days 1, 3, and 5. The severity of psoriasiform lesions (thickening, scaling, and erythema) was evaluated using PASI scores. The skin of mice in the Vaseline group showed no psoriasis-like lesions (Figure 1 A). However, typical symptoms of scaling, thickness, and erythema were observed in the IMQ and IMQ/IL-36γ groups (Figures 1 B, C). Furthermore, the total PASI scores in the IMQ/IL-36γ group (7.33 ±2.07 vs. 9.83 ±1.33, p = 0.015 at day 5, 6.42 ±1.63 vs. 9.42 ±1.16,

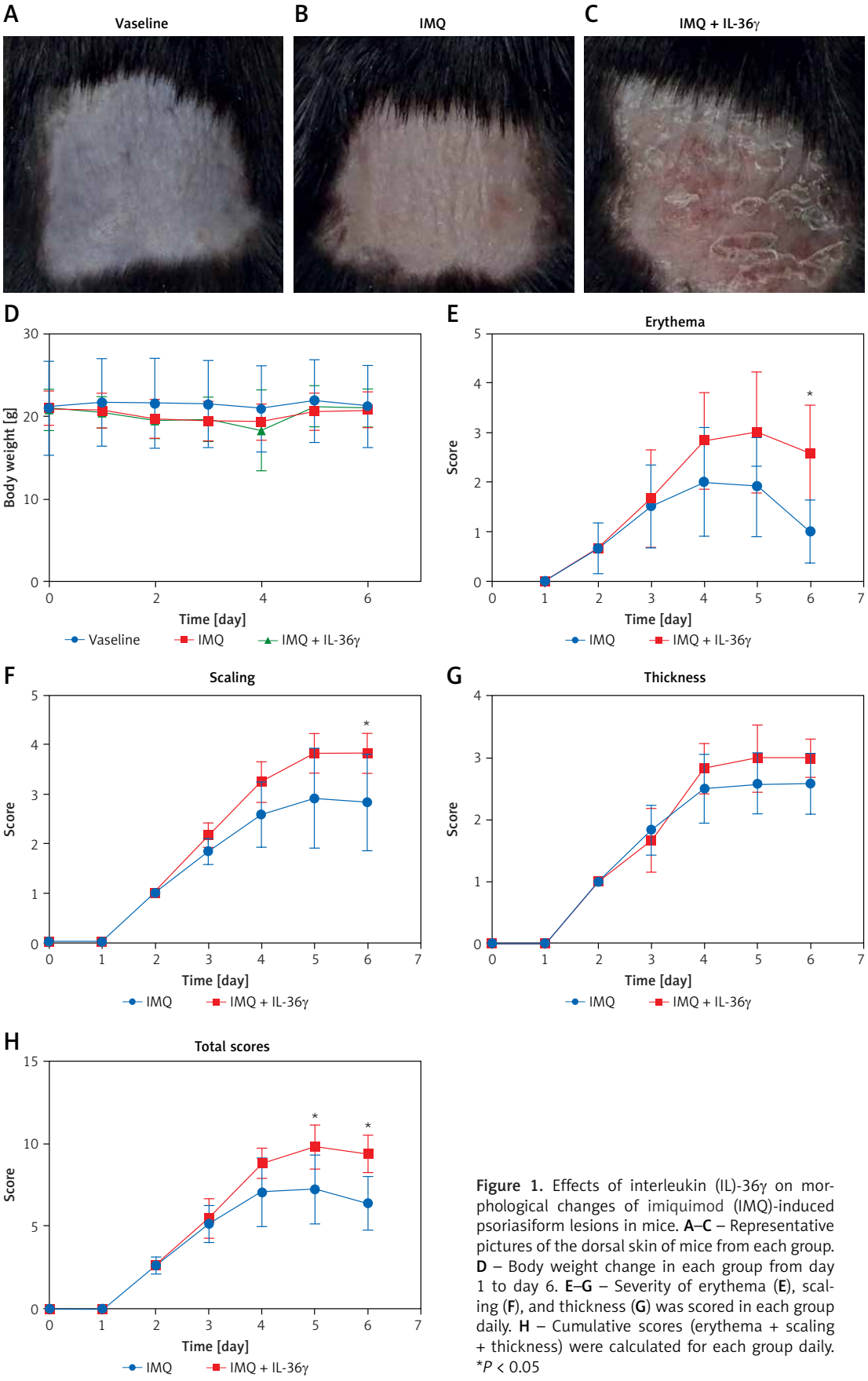

Figure 1. Effects of interleukin (IL)-36γ on morphological changes of imiquimod (IMQ)-induced psoriasiform lesions in mice. **A–C** – Representative pictures of the dorsal skin of mice from each group. **D** – Body weight change in each group from day 1 to day 6. **E–G** – Severity of erythema (**E**), scaling (**F**), and thickness (**G**) was scored in each group daily. **H** – Cumulative scores (erythema + scaling + thickness) were calculated for each group daily. *$P < 0.05$

p = 0.010 at day 6) were higher than those in the IMQ group (Figures 1 E–H). These results indicated that the clinical symptoms of psoriasiform lesions induced by IMQ treatment in mice can be significantly exacerbated by IL-36γ.

The histopathological characteristics of psoriasiform lesions were aggravated in IL-36γ-deficient mice following treatment with IL-36γ

H&E staining was used to evaluate the histopathological characteristics of the dorsal lesions in mice of the three experimental groups (Vaseline, IMQ, and IMQ/IL-36γ). Mice treated with IMQ and IMQ/IL-36γ showed various pathologic changes characteristic of psoriasis including epidermal hyperplasia, thickening of the acanthosis cell layer, inflammatory cell infiltration, and increased numbers of blood vessels (Figures 2 A–F). Epidermal thicknesses in the IMQ and IMQ/IL-36γ groups were 32.33 ±5.84 and 47.83 ±3.25 µm, respectively, compared with only 22.98 ±5.94 µm in the Vaseline group. Epidermal thickness in the IMQ/IL-36γ group was significantly higher than that in the IMQ group (Figure 2 G). Numbers of blood vessels and total cells in the dermis were higher in the IMQ and IMQ/IL-36γ groups compared with the Vaseline group. Numbers of blood vessels and total cells in the dermis were higher in the IMQ/IL-36γ group compared with the IMQ group (Figures 2 H, I).

IL-36γ attenuates the expression of loricrin and keratin 5 in IL-36γ-deficient mice

To investigate the effects of IL-36γ on keratinocyte differentiation, expression of loricrin, keratin 5 and keratin 17 in mouse skin lesions was assessed by immunohistochemistry. Semi-quantitative staining showed that loricrin expression was significantly lower in the IMQ and IMQ/IL-36γ groups compared with the control group (Figures 3 A–D). Additionally, expression of loricrin and keratin 5 in skin lesions was decreased following treatment with IL-36γ (Figures 3 E–H). However, expression of keratin 17 was negative in the three groups of mice. These results demonstrated that IL-36γ treatment induced abnormal differentiation of keratinocytes following administration of IMQ (Figures 3 I–K).

IL-36γ induces psoriasis-associated IL-17A and IFN-γ expression in IL-36γ-deficient mice

We next investigated the effects of IL-36γ on expression of inflammatory mediators (IL-6, IL-17A, and IFN-γ) in the skin lesions of mice by immunohistochemistry.

IMQ exposure significantly increased the expression of IL-6, IL-17A, and IFN-γ. The markedly increased expression of IL-17A and IFN-γ, but not of IL-6, was further elevated following IL-36γ treatment (Figure 4). These data suggested that IL-36γ may exacerbate the inflammatory reactions of keratinocytes in a murine model of psoriasis.

Expression of β-catenin was upregulated in IL-36γ-deficient mice following treatment with IL-36γ

Expression levels of β-catenin and Dickkopf-related protein 1 (DKK1) in mice were assessed. As shown in Figure 5, β-catenin and DKK1 expression was elevated following IMQ stimulation (Figures 5 A, B, E, F), and increased expression was enhanced by IL-36γ treatment (Figures 5 C, D, G, H). These results indicated that Wnt pathway signaling may mediate the effects of IL-36γ on IMQ-induced psoriasis.

Discussion

Psoriasis is a common, environmentally influenced, chronic inflammatory skin disorder affecting around 2–3% of the population. IL-36γ is mainly restricted to keratinocytes and can contribute to skin inflammation by acting on keratinocytes and antigen-presenting cells [7, 8]. Previous studies showed that IL-36γ levels were significantly higher in lesions from psoriasis patients compared with healthy skin [8]. Using a murine model of IMQ-induced psoriasis and IL-36γ-deficient mice, we investigated the effects of IL-36γ on the epidermis in psoriatic lesions. Our results demonstrated that IL-36γ exerted a positive effect on keratinocytes during the pathogenesis of psoriasis, promoting epidermal hyperplasia, inflammatory cell infiltration, increased vascularization, inhibition of differentiation, and pro-inflammation cytokine secretion.

The role of IL-36Ra in psoriasis has been previously studied. Treatment with IL-36Ra reduced the severity of IMQ-induced psoriasiform lesions in mice. BI 655130, a monoclonal antibody against the interleukin-36 receptor, has been used for the treatment of generalized pustular psoriasis [9]. Consistent with the results of these studies, we demonstrated that IL-36γ can exacerbate the clinical phenotype of IMQ-induced psoriatic dermatitis in IL-36γ-deficient mice. IL-36γ administration significantly increased the epidermal thickness of IMQ-induced psoriasiform lesions. These data suggested that IL-36γ may play an important role in the pathogenesis of psoriasis.

Although psoriasis is considered an immune-mediated disease, immune cells do not function in isolation. The interactions among im-

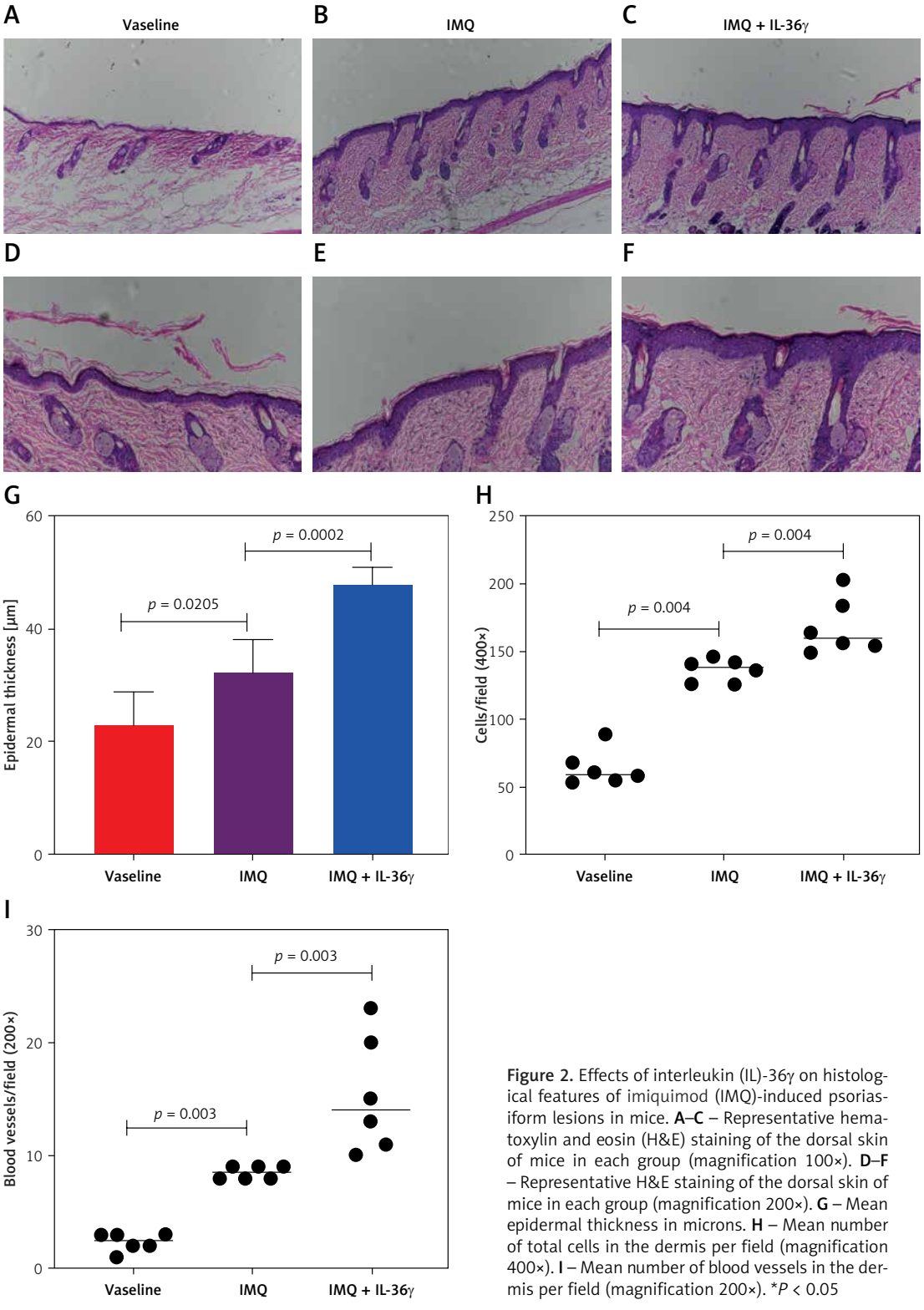

Figure 2. Effects of interleukin (IL)-36γ on histological features of imiquimod (IMQ)-induced psoriasiform lesions in mice. **A–C** – Representative hematoxylin and eosin (H&E) staining of the dorsal skin of mice in each group (magnification 100×). **D–F** – Representative H&E staining of the dorsal skin of mice in each group (magnification 200×). **G** – Mean epidermal thickness in microns. **H** – Mean number of total cells in the dermis per field (magnification 400×). **I** – Mean number of blood vessels in the dermis per field (magnification 200×). *$P < 0.05$

mune cells, keratinocytes, and cytokines are also involved in the pathogenesis of psoriasis. Keratinocytes are the main cells of the skin barrier. Skin keratinocytes undergo sequential physiological processes including proliferation, differentiation, and cell death. Each process is characterized by the expression of different markers [10]. Previous studies revealed that the skin barrier was impaired in psoriasis. Loricrin has an important role in forming the cornified envelope. Patients with loricrin mutations develop hyperkeratosis, which can also be seen in psoriasis lesions.

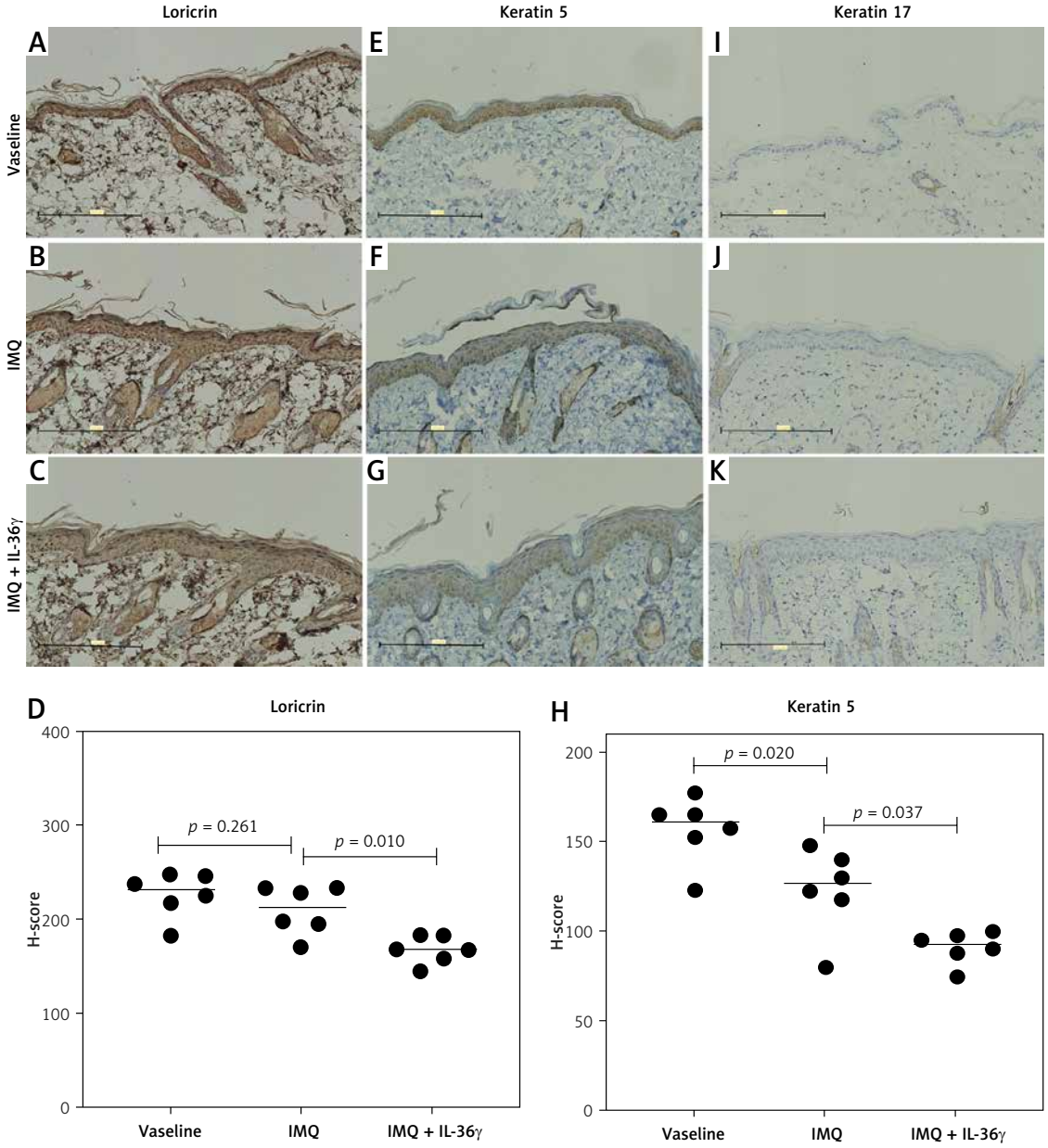

Figure 3. Interleukin (IL)-36γ inhibited epidermal differentiation in an imiquimod (IMQ)-induced mouse model of psoriasis. Expression of loricrin (**A**–**C**), keratin 5 (**E**–**G**) and keratin 17 (**I**–**K**) was assessed in the epidermis of mice from each group (scale bars 200 µm). **D**, **H** – Mean H-scores for loricrin and keratin 5, respectively, in the epidermis of mice from each group. *$P < 0.05$

Previous studies also showed that expression of loricrin was downregulated in the lesions of psoriasis patients and could be enhanced by treatment with tumor necrosis factor-α antagonists [11]. Keratin 5 is the main protein in basal keratinocytes [10]. Our study found that expression of loricrin and keratin 5 in psoriasis lesions was downregulated following IL-36γ treatment. Keratin 17 is an epithelial keratin and plays a role in the integrity of the epidermis. Keratin 17 is considered a marker of keratinocyte proliferation. After certain exogenous stressors, such as skin injury, keratin 17 can be induced in keratinocytes. Previous studies demonstrated that keratin 17 was expressed in the lesions of psoriasis patients and that its expression was positively associated with psoriasis severity. Furthermore, the expression of keratin 17 represents the highly activated and proliferative status of the epidermis in psoriasis [12]. These studies indicated that K17 may play an important role in the onset stage of psoriasis. K17 is considered an autoantigen for autoreactive T cells and the K17/T-cell/cytokine autoimmune loop is implicated in the pathogenesis of psoriasis. However, a study also showed that K17 expression induced by IL-17A

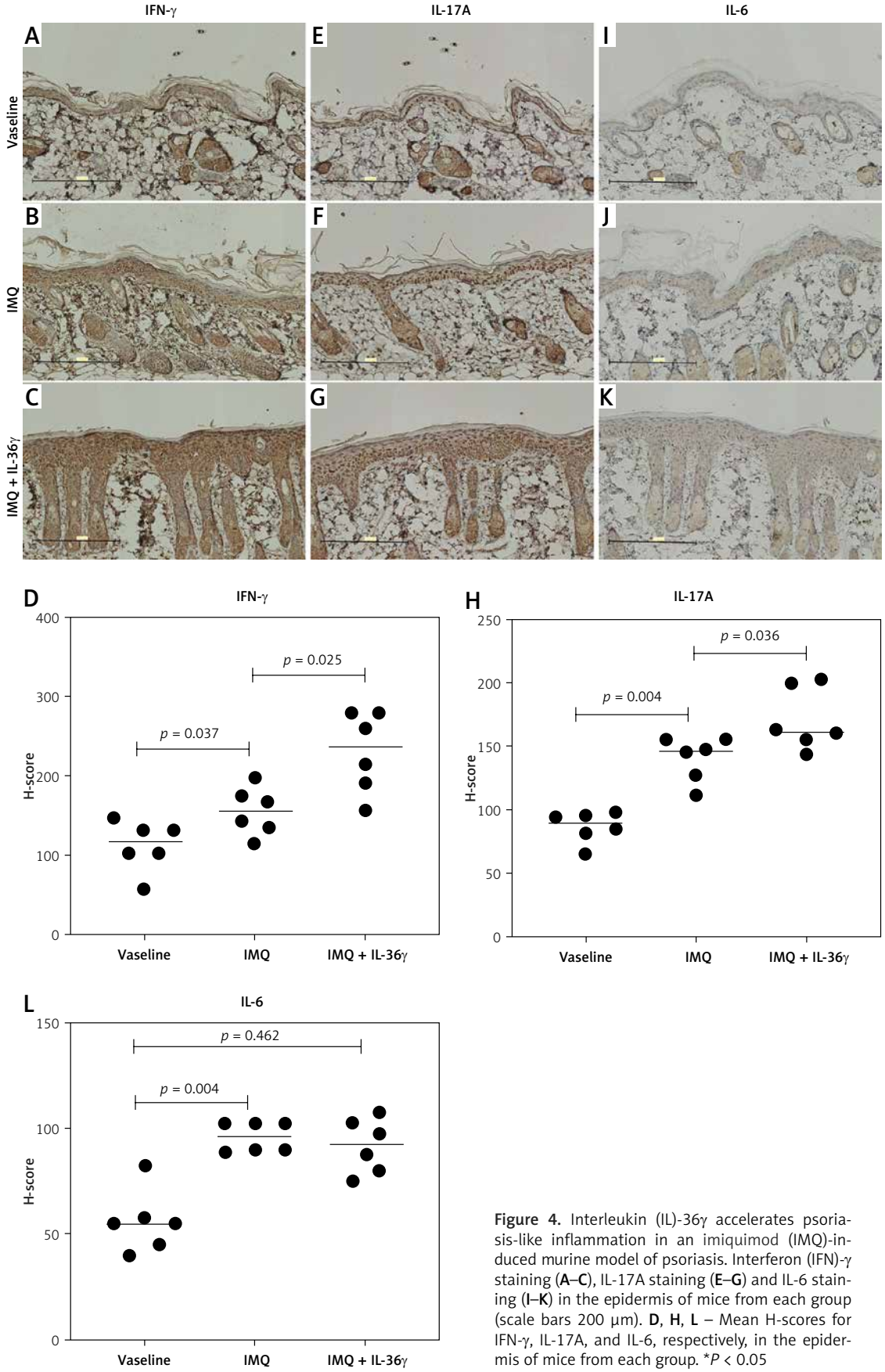

Figure 4. Interleukin (IL)-36γ accelerates psoriasis-like inflammation in an imiquimod (IMQ)-induced murine model of psoriasis. Interferon (IFN)-γ staining (**A**–**C**), IL-17A staining (**E**–**G**) and IL-6 staining (**I**–**K**) in the epidermis of mice from each group (scale bars 200 µm). **D**, **H**, **L** – Mean H-scores for IFN-γ, IL-17A, and IL-6, respectively, in the epidermis of mice from each group. *$P < 0.05$

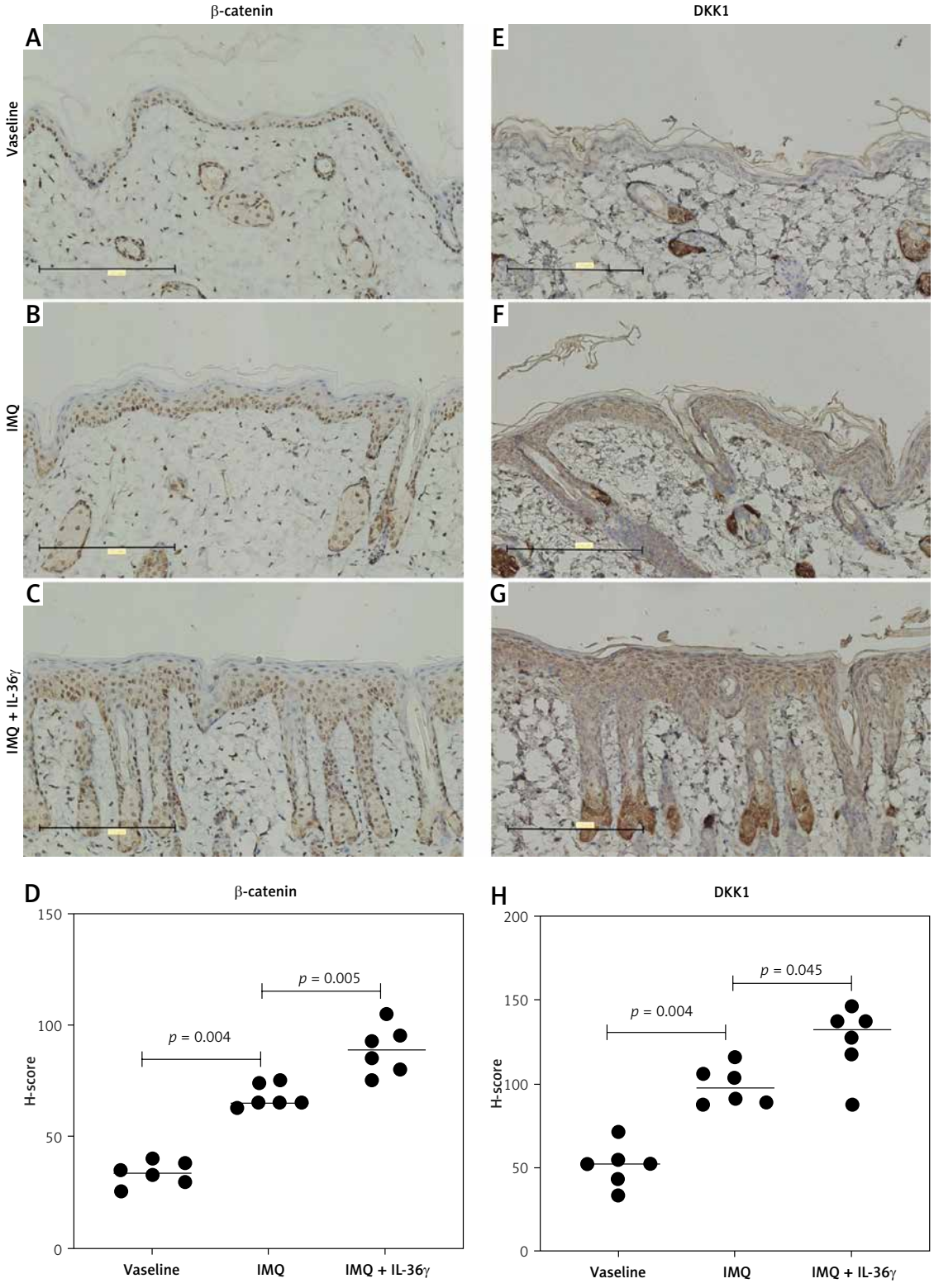

Figure 5. Effects of interleukin (IL)-36γ on the β-catenin signaling pathway in imiquimod (IMQ)-induced murine psoriasiform lesions. Expression of β-catenin (**A**–**C**) and DKK1 (**E**–**G**) was assessed in the epidermis of mice from each group (scale bars 200 μm). **D**, **H** – Mean H-scores for β-catenin and DKK1, respectively, in the epidermis of mice from each group. *$P < 0.05$

in keratinocytes was dose-dependent [13]. In this study, we observed no expression of keratin 17 in any of the three groups of mice studied here. We speculate that expression of keratin 17 may be related to stage of psoriasis (e.g., the acute or chronic stage) and the psoriatic lesions of mice in our study were in the stable stage of psoriasis. Our results suggest that IL-36γ may be involved in the abnormal differentiation of keratinocytes and play a role in the pathogenesis of psoriasis.

Keratinocytes are the major constituents of the epidermis and act as the trigger and the executor in the pathogenesis of psoriasis. Keratinocytes are crucial in forming a physical barrier and are also major sources of inflammatory cytokines [14]. IL-17A is the main member of the IL-17 family and contributes to the pathogenesis of several diseases including psoriasis. Anti-IL-17A monoclonal antibodies, such as secukinumab and ixekizumab, can significantly alleviate psoriasis disease activity compared with placebo. IFN-γ was recognized for its antiviral activity and is released by activated T cells and natural killer cells [15]. Previous work showed that in inflammatory settings, IFN-γ could induce autocrine IFN-γ production by keratinocytes [16]. Our results showed that the staining intensity of IL-17A and IFN-γ in keratinocytes was enhanced following IL-36γ stimulation. This finding indicated that IL-36γ may be involved in innate immune system dysfunction in psoriasis. IL-6 can be expressed by human keratinocytes and promotes the proliferation of keratinocytes. Anti-IL-6 therapies were effective for patients with psoriatic arthritis [17]. Our study demonstrated that the staining intensity of IL-6 was not significantly different between the IMQ and IMQ/IL-36γ groups. In agreement with previous studies, our results suggested that IL-36γ may be involved in imbalanced innate and adaptive immunity in psoriasis.

β-catenin is a 94 kDa protein that functions as a transcription factor of the Wnt signaling pathway. DKK-1 is an inhibitor of the canonical Wnt pathway [18]. Nuclear β-catenin expression was found to be enhanced in the lesions of psoriasis patients, while membranous β-catenin expression was lower in the lesions of psoriasis patients compared with controls [19]. However, another study showed the expression of β-catenin was lower in lesions from psoriasis patients compared with healthy controls [20]. Our study found that expression of β-catenin and DKK1 was elevated in an IMQ-induced murine model of psoriasis, and that increases in expression were enhanced by IL-36γ treatment. We speculate that upregulation of DKK1 may represent feedback from the increase in β-catenin expression. Our findings indicate that β-catenin may mediate the role of IL-36γ in psoriasis, a possibility that will need to be clarified in further studies.

There are limitations of our study. First, our study was focused on the role of IL-36γ in an imiquimod-induced murine model of psoriasis and was not mainly designed to study the role of IL-36γ in mice. Therefore, the mice treated only with IL-36γ were not included in this study. In fact, adding the data of the mice treated only with IL-36γ can elucidate the function of IL-36γ more comprehensively. Second, our study describes a model of psoriasis in IL-36γ-deficient mice, but the pathogenesis is not addressed in wild type mice. Future mechanistic work should address the role of IL-36γ in wild type mice, which may facilitate elucidation of the mechanisms of IL-36γ in the pathogenesis of psoriasis.

In conclusion, our results suggested that IL-36γ plays a role in keratinocyte activation in psoriasis. Uncontrolled keratinocyte proliferation, aberrant differentiation of keratinocytes, and inflammatory cytokine secretion were detected following IL-36γ treatment in an IMQ-induced murine model of psoriasis. These findings suggest that IL-36γ may represent a biomarker of psoriasis and suggest new directions toward development of anti-IL-36R treatments for psoriasis patients. The role of β-catenin in the function of IL-36γ in psoriasis requires further study.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

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