

Interleukin-18 exacerbates skin inflammation and affects microabscesses and scale formation in a mouse model of imiquimod-induced psoriasis

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Abstract

Background: As a potent pro-inflammatory cytokine of the interleukin (IL)-1 family, IL-18 was elevated in early active and progressive plaque-type psoriatic lesions and that serum or plasma levels of IL-18 correlated with the Psoriasis Area and Severity Index (PASI). Although results from previous studies have established that IL-18 may aggravate psoriatic inflammation, the mechanisms of this process remain unknown. In this study, *IL-18* knock out (KO) mice and wild-type (WT) mice were used to investigate the effects of IL-18 within a mouse model of psoriasis.

Methods: WT and *IL-18* KO mice were divided into four groups, including imiquimod (IMQ)-treated *IL-18* KO group ($n=11$) and WT group ($n=13$) as well as their respectively gene-matched control mice (receiving vaseline; $n=12$). PASI scores were used to evaluate psoriatic lesions in IMQ-treated mice. Pathological features and dermal cellular infiltration were investigated by hematoxylin and eosin staining. The levels of psoriasis-related cytokines including IL-23, IL-17, IL-12, IL-1 β , IFN γ , IL-15, IL-27, and IL-4 were tested by real-time polymerase chain reaction (PCR). The protein level of IL-1 β , IL-27, CXCL1, and Ly6g were investigated by immunohistochemistry (IHC).

Results: Acanthosis (98.46 ± 14.12 vs. 222.68 ± 71.10 μm , $P < 0.01$) and dermal cell infiltration (572.25 ± 47.45 vs. 762.47 ± 59.59 cells/field, $P < 0.01$) were significantly milder in IMQ-induced *IL-18* KO mice compared with that in WT mice. IMQ-induced *IL-18* KO mice manifested larger areas of Munro microabscesses ($11,467.83 \pm 5112.09$ vs. 4093.19 ± 2591.88 μm^2 , $P < 0.01$) and scales ($100,935.24 \pm 41,167.77$ vs. $41,604.41 \pm 14,184.10$ μm^2 , $P < 0.01$) as compared with WT mice. In skin lesions of *IL-18* KO mice, the expressions of IL-1 β , IL-4, and IL-27 were all significantly upregulated but IL-17 was decreased. Histologically, strong positive signals of Ly6g were observed within the epidermis of *IL-18* KO mice but expressions of CXCL1 were decreased.

Conclusions: IL-18 may exacerbate prominent inflammation and influence pathological features in IMQ-induced mouse model of psoriasis. IL-18 may upregulate pro-inflammatory cytokines and reduce protective cytokines, thus aggravating psoriatic inflammation. In addition, IL-18 may be involved in the formation of Munro microabscesses and scales.

Keywords: Interleukin-18; Psoriasis; Cytokines; Scales

Introduction

Psoriasis is a common chronic inflammatory skin disease that affects 2% to 3% of the population worldwide.^[1] Its etiology is unknown, but it is believed to involve a complex network of cytokines and chemokines produced by various types of immune and tissue cells.^[2] Although the pathogenesis of psoriasis is not fully understood, there is growing evidence which indicates that T helper 1 (Th1) and 17 (Th17) cells play a critical role in the development of this disease.^[3]

Interleukin (IL)-18, is a potent pro-inflammatory cytokine of the IL-1 family.^[4] When synergized with IL-23, IL-18 promotes the development and maintenance of Th17 cells that have been implicated in autoimmune inflammatory diseases.^[5,6] IL-18 also plays an important role in activating Th1 cells which can then produce interferon γ (IFN γ) mediated inflammation in psoriatic lesions.^[7]

Results from previous studies have indicated that serum or plasma levels of IL-18 correlate well with the Psoriasis Area and Severity Index (PASI).^[8,9] The expression of

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DOI:
10.1097/CM9.0000000000000140

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Chinese Medical Journal 2019;132(6)

Received: 05-11-2018 Edited by: Yuan-Yuan Ji

IL-18 was elevated in initially active and progressive plaque-type psoriatic lesions.^[10] Moreover, in cooperation with IL-23, IL-18 induced prominent inflammation and enhanced psoriasis-like epidermal hyperplasia.^[11] Although it has been clearly demonstrated that IL-18 may aggravate psoriatic inflammation, the specific mechanisms of this process remain unknown. Few studies have been directed toward investigating the means through which IL-18 affects disease severity. In this report, *IL-18* knockout (KO) mice were used to investigate the effects of IL-18 within a mouse model of psoriasis-like skin inflammation as induced by imiquimod.

Methods

Mice and treatment

C57BL/6 and *IL-18* KO (C57BL/6 background) mice were obtained from the Jackson Laboratory (stock No. C57BL/6: 000664-JAX; *IL-18* KO: 004130-JAX, Bar Harbor, ME, USA). These mice were bred in house within a pathogen free animal facility and allowed plenty of water and food. Mice were divided into four groups: wild-type (WT) control group ($n=12$), *IL-18* KO control group ($n=12$), WT + imiquimod (IMQ) group ($n=13$), and *IL-18* KO + IMQ group ($n=11$). All mice were shaved on their dorsal surface to expose a 2 cm × 3 cm area. The IMQ-treated mice received a daily application of IMQ using a dose of 62.5 mg IMQ cream (5%, Aldara, 3M, USA).^[12] The control mice received an identical volume of vaseline cream. A sample of skin was collected from all mice at 8 days after treatment.

All experimental procedures in this study abided by the *Guide for the Care and Use of Laboratory Animals* (NIH Publication, 8th Edition, 2011) and were conducted according to the guidelines provided by the Institutional Animal Care and Use Committee at China Medical University (IACUC No. 16008M).

Scoring severity of skin inflammation

To score the severity of IMQ-induced inflammation an objective scoring system was developed based on the

clinical PASI. Erythema, scaling and thickening were scored independently from 0 to 4 as follows: 0 = none, 1 = slight, 2 = moderate, 3 = marked and 4 = very marked. The cumulative score (erythema plus scaling plus thickening) served to indicate the severity of inflammation (cumulative scale = 0–12). The scoring process was performed by three investigators.

Real-time polymerase chain reaction

Total RNA was extracted from whole biopsies of the exposed skin from the dorsal area of euthanized mice using a miRNeasy mini kit (Qiagen, Germany). The mRNA was then transcribed to cDNA by the GoScriptTM Reverse Transcription System (Promega, USA). The sequences of primers are shown in Table 1. Real-time polymerase chain reaction (PCR) was performed in 384-well plates with use of a 7900HT Fast Real-Time PCR system (Applied Biosystems Industries, Foster city, USA). cDNA (1 μL) was mixed with 5-μL GoTaq[®] qPCR Master Mix, 0.1-μL CXR Reference Dye, 0.4-μL primer, and 3.5-μL Nuclease-free water to a final volume of 10 μL. The Ct value was calculated using RQ Manager Software (Applied Biosystems Industries, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the internal control and expression levels of target genes were calculated by applying $2^{-\Delta\Delta CT}$ methods. The experiments were repeated three times.

Hematoxylin and eosin staining

Hematoxylin and eosin (HE) staining was performed using standard procedures. Each section subjected to HE staining was repeatedly microphotographed using light microscopy (original magnification, ×200) to achieve quality control. Three microphotographs (the first, middle, and last fields) were then chosen to assess psoriasis-like skin lesions in each sample using Image-pro Plus software 6.0 (Media Cybernetics, Rockville, USA).^[13] The specific items for assessment included: (1) Measurement of acanthosis thickness, (2) measurement area of scales, (3) measurement area of Munro microabscesses, and (4) counting of cells within the dermal layer.

Table 1: The Primer sequences for quantitative real-time PCR.

Genes	Forward primer sequence	Reverse primer sequence
GAPDH	5'-GGGCTCTGCTCCTCCCTGT-3'	5'-CGGCCAAATCCGTTACACCCG-3'
IL-1β	5'-CACTACAGGCTCCGAGATGAA-3'	5'-TGTCGTTGCTTGGTTCTCCT-3'
IFNγ	5'-ATGAACGCTACACACTGCATC-3'	5'-CCATCCTTTTGCCAGTTCCTC-3'
IL-27	5'-CTGTTGCTGCTACCCTTGCTT-3'	5'-CACTCCTGGCAATCGAGATTTC-3'
IL-17A	5'-ACCGCAATGAAGACCCTGATA-3'	5'-TCTTCTCGACCCTGAAAGTGAA-3'
IL-17F	5'-ACCCGTGAAACAGCCATGGTCAAG-3'	5'-CCCATGGGGAAGTGGAGCGG-3'
IL-4	5'-GAGACTCTTTCGGGCTTT-3'	5'-ATGCTCTTTAGGCTTTCCA-3'
IL-15	5'-ACATCCATCTCGTGCTACTTGT-3'	5'-GCCTCTGTTTTAGGGAGACCT-3'
IL-18R	5'-GAAGAAGCCATAGACACCAAGAG-3'	5'-AGGCGAGAACAAGCACAGT-3'
IL-23A	5'-ACCTGCTTGACTCTGACATCTT-3'	5'-CTGCCACTGCTGACTAGAACT-3'
IL-12A	5'-CTGGAACCTACACAAGAACGAGAG-3'	5'-GGCACAGGGTCATCATCAAAG-3'

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; IFN: Interferon; IL: Interleukin; PCR: Polymerase chain reaction.

Measurements of microphotographs were then averaged to calculate each sample's mean value for all four items. Some processes of measurements were manually performed (semi-automated). Two independent investigators who were blind as to each other's measurements and treatment groups of the mice performed these analyses.

Immunohistochemistry

Immunohistochemistry was performed with use of a BenchMark[®]GX Automated Slide Stainer (Roche, Switzerland, sequence No. 815700). Tissue sections were incubated with IL-1 β polyclonal rabbit antibody (dilution 1:500, cat.no.ab9722, Abcam, UK), mouse CXCL1 rabbit polyclonal antibody (dilution 1:200, cat.no.ab86436, Abcam), mouse Ly6g rat monoclonal antibody (dilution 1:100, cat.no.ab25377, Abcam), and mouse IL-27 rabbit polyclonal antibody (dilution 1:200, cat.no.GTX54307, GenTex, USA). Image Pro-plus 6.0 software (Media Cybernetics, USA) was used to assess immunohistochemical sections by measuring the integrated optical density (IOD).

Statistical analysis

The tested variables with a normal distribution were presented as mean \pm standard deviation (SD), and difference between two groups were compared as using of Student's *t* test. Otherwise, variables with skewed distributions were expressed as median (Q₁, Q₃), and differences between the analysis groups were compared using the Kruskal-Wallis test or Mann-Whitney *U* test. A statistical significance was defined as *P* < 0.05. All analyses were performed by SPSS software (version 19.0, IBM SPSS, Inc. Chicago, IL, USA).

Results

IL-18 is associated with extent of skin lesions and PASI scores and enhances IMQ-induced inflammation and acanthosis

IMQ-induced skin lesions in WT mice showed evidence of erythema and epidermal thickness with silvery thick scales, whereas no obvious skin lesions were present in *IL-18* KO mice [Figure 1A]. As based upon PASI scores, the skin of IMQ-induced WT mice began to display signs of thickness, erythema and scales on day 2. These symptoms continued from days 2 to 4, increased in severity up to day 7, but regressed on the eighth day. However, in IMQ-induced *IL-18* KO mice these symptoms continued to increase for 8 days [Figure 1B–D]. Cumulative PASI scores revealed that *IL-18* KO mice had significantly lower scores than WT mice on days 3 to 7 in response to IMQ (day 3: 0.00 [0.00, 0.50] vs. 4.00 [3.25, 4.00], *Z* = -3.248, *P* = 0.001; day 4: 1.67 \pm 0.52 vs. 4.50 \pm 1.19, *t* = -5.410, *P* < 0.001; day 5: 2.33 \pm 1.37 vs. 6.62 \pm 1.41, *t* = -5.714, *P* < 0.001; day 6: 3.83 \pm 1.47 vs. 8.38 \pm 1.30, *t* = -6.113, *P* < 0.001; day 7: 5.83 \pm 1.17 vs. 10.00 \pm 1.07, *t* = -6.939, *P* < 0.001; Figure 1E). Control mice showed no visible inflammation within the exposed areas of their dorsal surface [Figure 1A].

Histopathological evaluations of skin specimens obtained from mice treated with IMQ showed acanthosis and moderate cellular infiltration in the dermis as reported previously.^[11] IMQ-induced *IL-18* KO mice showed a milder degree of acanthosis (98.46 \pm 14.12 vs. 222.68 \pm 71.10 μ m, *t* = -4.500, *P* = 0.003) and cellular infiltration (572.25 \pm 47.45 vs. 762.47 \pm 59.59 cells/field, *t* = -5.182, *P* = 0.001) as compared with that observed in WT mice

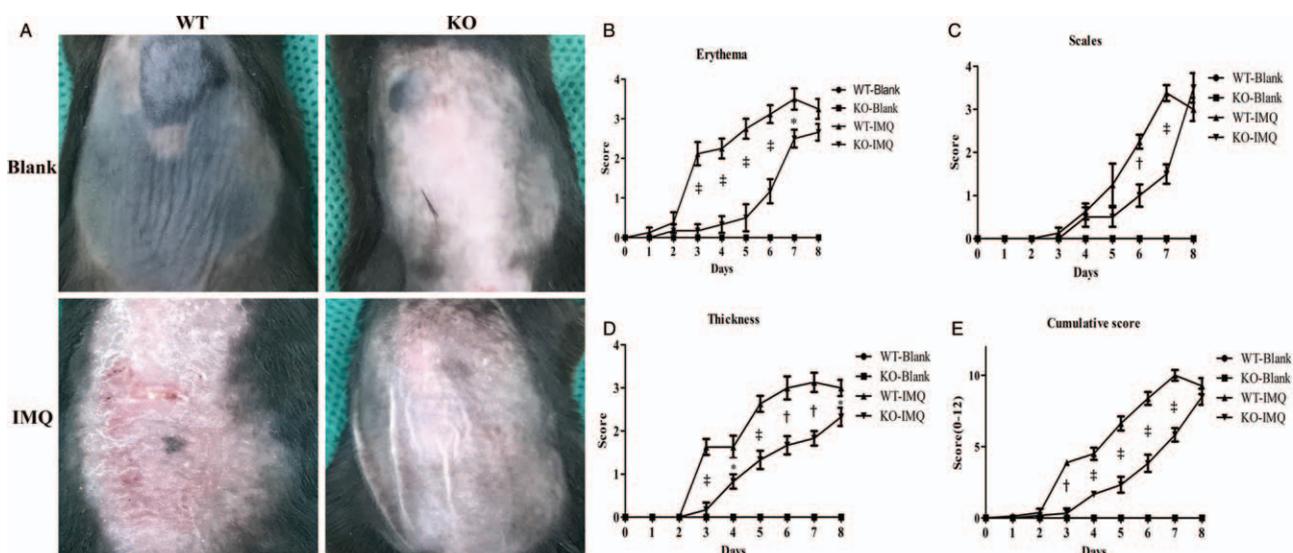


Figure 1: *IL-18* exacerbated IMQ-induced psoriasis-like skin inflammation and was associated with Psoriasis Area and Severity Index (PASI). (A) Macroscopic phenotypical representation of dorsal skin lesions in all groups as performed on the eighth day after IMQ treatment. (B) Erythema of dorsal skin in WT-Blank, KO-Blank, IMQ-WT, and IMQ-KO groups was scored (0–4) on the indicated days. (C) Scaling scores in four groups. (D) Thickness scores in four groups. (E) The cumulative scores (0–12: erythema plus scaling plus thickness) are presented. [†]*P* < 0.05, [‡]*P* < 0.01, [§]*P* < 0.001, IMQ-induced WT mice vs. *IL-18* KO mice. Blank: Control mice; IMQ: Imiquimod; KO: *IL-18* knockout mice; WT: Wild-type mice.

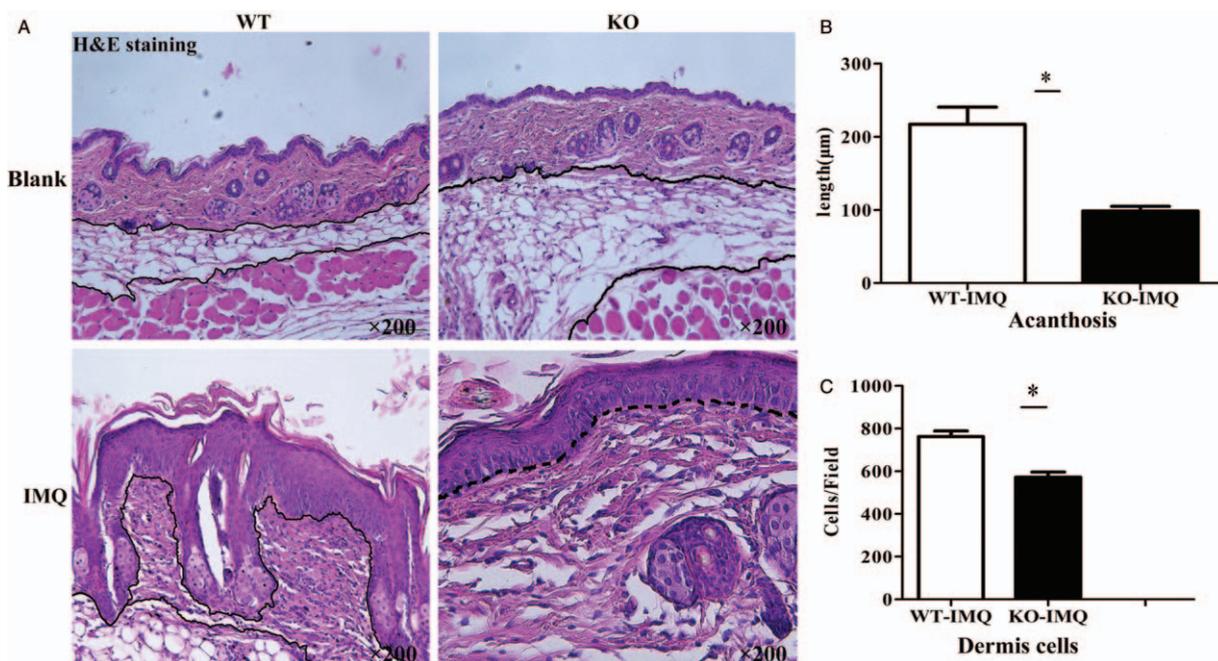


Figure 2: (A) Acanthosis and dermal cell infiltration were milder in IMQ-treated *IL-18* KO mice. H&E staining (original magnification $\times 200$) as performed on samples from the dorsal skin of mice within the different treatment groups. (B) The measurement of acanthosis thickness was performed on IMQ-induced mice, and KO mice showed a milder degree of acanthosis than WT mice. (C) Total cell numbers within a single field of HE sections of IMQ-treated mice are shown. IMQ-treated KO mice showed significantly less cell infiltration than WT mice. The black line indicates the boundary among the epidermis and dermis and subcutaneous. $P < 0.01$, IMQ-induced WT mice vs. *IL-18* KO mice. Blank: Control mice; IMQ: Imiquimod; KO: *IL-18* knockout mice; WT: Wild-type mice.

[Figure 2]. The epidermis of control mice contained only 1 to 2 layers of cells and there was no infiltration of inflamed cells within the dermal layer of these mice.

IL-18 is associated with Munro microabscesses and scale formation in IMQ-induced skin inflammation

Munro microabscesses, as a characteristic hallmark of psoriasis pathology, are reported to be located in the epidermis and contain substantial numbers of neutrophils.^[14] Here, we found that Munro microabscesses ($11,467.83 \pm 5112.09$ vs. $4093.19 \pm 2591.88 \mu\text{m}^2$, $t = 3.309$, $P = 0.008$) and scales ($100,935.24 \pm 41,167.77$ vs. $41,604.41 \pm 14,184.10 \mu\text{m}^2$, $t = 3.586$, $P = 0.005$) were present in a significantly greater amount of area in *IL-18* KO vs. WT mice in response to IMQ [Figure 3].

IL-18 upregulates mRNA levels of pro-inflammatory cytokines but downregulates protective cytokines and IL-1 β

To determine some of the potential mechanisms underlying the milder psoriasis-like skin inflammation of *IL-18* KO mice, we analyzed the profiles of cytokines that have been reported to be involved in the pathogenesis of psoriasis. RT-PCR analysis showed that expressions of *IL-1 β* (343.04 [220.69, 459.12] vs. 54.99 [13.87, 179.90], $Z = -2.021$, $P = 0.043$), *IL-27* (1360.68 [985.52, 2549.14] vs. 373.15 [47.85, 1011.42], $Z = -2.415$, $P = 0.016$) and *IL-4* (6276.45 [3811.54, 7510.34] vs. 1312.03 [207.67, 3524.04], $Z = -2.882$, $P = 0.004$) were all significantly increased in IMQ-induced *IL-18* KO as compared to WT mice [Figure 4A]. *IL-17* was decreased in *IL-18* KO vs. WT mice (89.29 ± 30.06 vs. 188.34 ± 62.64 , $t = -2.851$,

$P = 0.029$, Figure 4A). The mRNA levels of *IFN γ* , *TNF α* , *IL-23*, *IL-12*, *IL-15*, and *IL-18R* were not significantly different between IMQ-induced *IL-18* KO and WT mice. Immunohistochemical assays were then performed to clarify protein expression levels of *IL-1 β* and *IL-27*. The results of these assays showed that in response to IMQ treatment, significantly increased expressions of *IL-1 β* (0.61 [0.53, 0.64] vs. 0.02 [0.01, 0.26], $Z = -2.661$, $P = 0.009$) and *IL-27* (0.59 ± 0.18 vs. 0.08 ± 0.10 , $t = 5.082$, $P = 0.002$) were obtained in *IL-18* KO compared to WT mice [Figure 5]. Accordingly, these results were consistent with those of the mRNA analysis.

IL-18 promotes production of CXCL1 and inflammatory forms of neutrophils

As an obvious cellular infiltration was found within the dermis, immunohistochemistry was used to measure expression of the chemokine, CXCL1. Compared with IMQ-treated WT mice, significantly lower expression levels of CXCL1 were present in *IL-18* KO mice (0.19 ± 0.01 vs. 0.75 ± 0.14 , $t = -6.943$, $P = 0.019$, Figure 5). Based upon these findings we presumed that neutrophil infiltration may be decreased in *IL-18* KO mice. As Ly6g is a surface protein that is expressed predominantly on neutrophils, it is considered to serve as a marker for neutrophils in mice.^[15] We found that IMQ-induced *IL-18* KO mice had significantly increased expressions of Ly6g within the epidermis as compared with that of WT mice (0.23 [0.13, 0.62] vs. 0.01 [0.00, 0.03], $Z = -2.739$, $P = 0.006$, Figure 5).

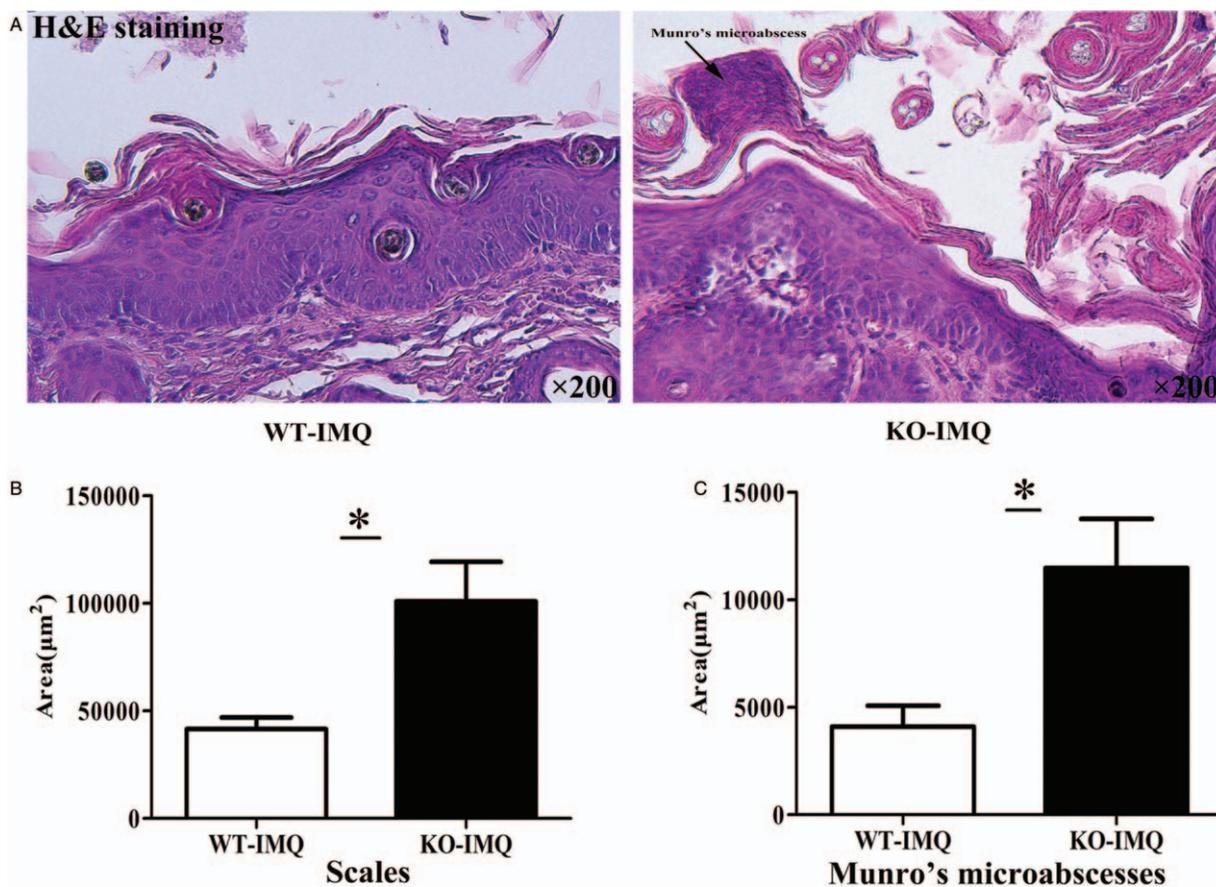


Figure 3: *IL-18* KO mice demonstrate larger areas of Munro microabscesses and scales. (A) H&E staining (original magnification $\times 200$) showed Munro microabscesses and Scales in response to IMQ. (B) The areas of scales were significantly increased in IMQ-induced KO mice vs. with WT mice. (C) The areas of Munro microabscesses were significantly increased in IMQ-induced KO mice vs. with WT mice. * $P < 0.01$. Blank: Control mice; IMQ: Imiquimod; KO: *IL-18* knockout mice; WT: Wild-type mice.

Discussion

The results of our study indicate that *IL-18* may aggravate psoriatic inflammation and the immunological effects of *IL-18* upon IMQ-induced psoriasis-like skin inflammation. Using an *IL-18* gene deficiency to establish a psoriatic mouse model, we found that IMQ-induced *IL-18* KO mouse showed milder psoriasis-like skin lesions, acanthosis and cellular infiltration compared with WT mice. *IL-18* might affect pathological features of psoriasis-like skin inflammation. In addition, *IL-18* promoted *IL-17* mRNA expression, while exerting negative effects on mRNA of *IL-1 β* , *IL-4*, and *IL-27*. *IL-18* might be involved in the formation of Munro microabscesses and scales, the specific mechanisms remain unknown.

This study represents to employ an *IL-18* deficient mouse model as an approach to examine the effects of *IL-18* in psoriasis. *IL-18*, as a member of the *IL-1* family of cytokines, plays a strong proinflammatory role in chronic inflammatory diseases. *IL-18* receptor (*IL-18R*) is a heterodimer, composed of α and β chains, and active *IL-18* can bind to either *IL-18R α* or *IL-18R β* . After forming the heterodimer, the intracellular Toll-*IL-1* receptor (TIR) domain binds to myeloid differentiation factor 88 (MYD88) and *IL-1* receptor-associated kinase (IRAK). Finally, the inflammatory pathway, nuclear

factor-kappa B (NF- κ B), would be activated by triggering a cascade of reaction.^[6,16]

IMQ, a Toll-like-receptor (TLR), 7/8 ligand and potent immune activator used for the treatment of actinic keratosis and superficial basal cell carcinomas.^[17] In 2009, van der Fits *et al*^[12] reported that application of imiquimod cream to the back skin of mice caused phenotypes with remarkable resemblance to human psoriasis pathology including the presence of Munro microabscesses. It was further established that the disease in mice was dependent upon the *IL-23/IL-17* axis. *IL-18* is considered as having a major role in the Th1 response, which induces Th1 cells to produce large amounts of $\text{IFN}\gamma$. Importantly, *IL-18*, also stimulates $\gamma\delta$ T and Th17 cells, which then secretes *IL-17*.^[3,16,18] Under Th1 conditions, *IL-18* can recruit dendritic cells expressing *IL-18R* and *IL-23* to areas of inflammation.^[6]

We presumed that *IL-18* might upregulate pro-inflammatory cytokine expression. As a result, *IFN γ* , *IL-23*, and *IL-17* mRNA would all be decreased within the skin lesions of *IL-18* KO mice. However, our results revealed that *IFN γ* and *IL-23* were not significantly downregulated within the skin lesions of these *IL-18* KO mice [Figure 4B]. Although *IL-18* can stimulate Th1 cells to produce large amounts of $\text{IFN}\gamma$ (results not shown), which appears to be important in early stage of psoriasis, its expression was

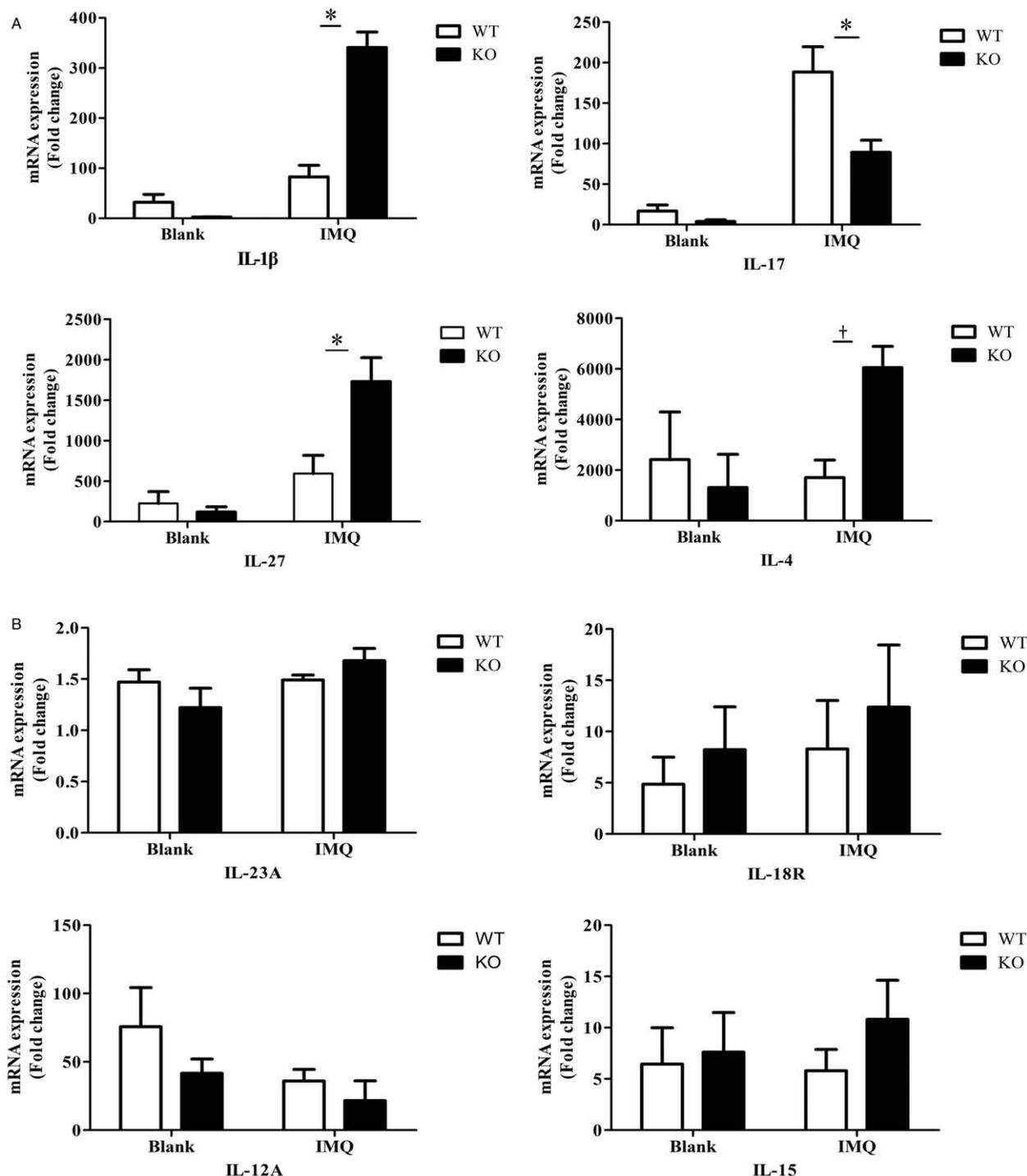


Figure 4: Levels of mRNA as determined using real time PCR. Mean of mRNA as determined on the eighth day after IMQ treatment in the different treatment groups. (A) mRNA levels of *IL-1 β* , *IL-27*, and *IL-4* were all significantly increased in IMQ-treated KO as compared to WT mice. *IL-17* was decreased in KO vs. WT mice. (B) mRNA levels of *IL-12A*, *IL-23A*, *IL-15*, and *IL-18R* were not statistically different between KO and WT mice. * $P < 0.05$, † $P < 0.01$. Blank: Control mice; IMQ: Imiquimod; KO: *IL-18* knockout mice; WT: Wild-type mice.

inhibited by Treg cells in plaque stage of psoriasis.^[3,4] In this study, *IFN γ* mRNA was tested at the eighth day after IMQ application, which showed no significant difference between *IL-18* KO mice and WT mice, possibly due to the tolerance of IMQ stimuli in such time. *IL-23* is a key molecule that promotes the development and maintenance of Th17 cells that have been implicated in autoimmune inflammatory diseases, especially psoriasis.^[19] Under

inflammatory conditions, myeloid dendritic cells producing *IL-23* are regulated by several pro-inflammatory cytokines including *IL-18*, *IL-1 β* , and *IFN γ* .^[20] It seems possible that *IL-18* is just one of the factors that contribute to the production of *IL-23* within dendritic cells.

IL-1 β , a pro-inflammatory cytokine of the *IL-1* family, possesses a sequence that highly resembles of *IL-18* and

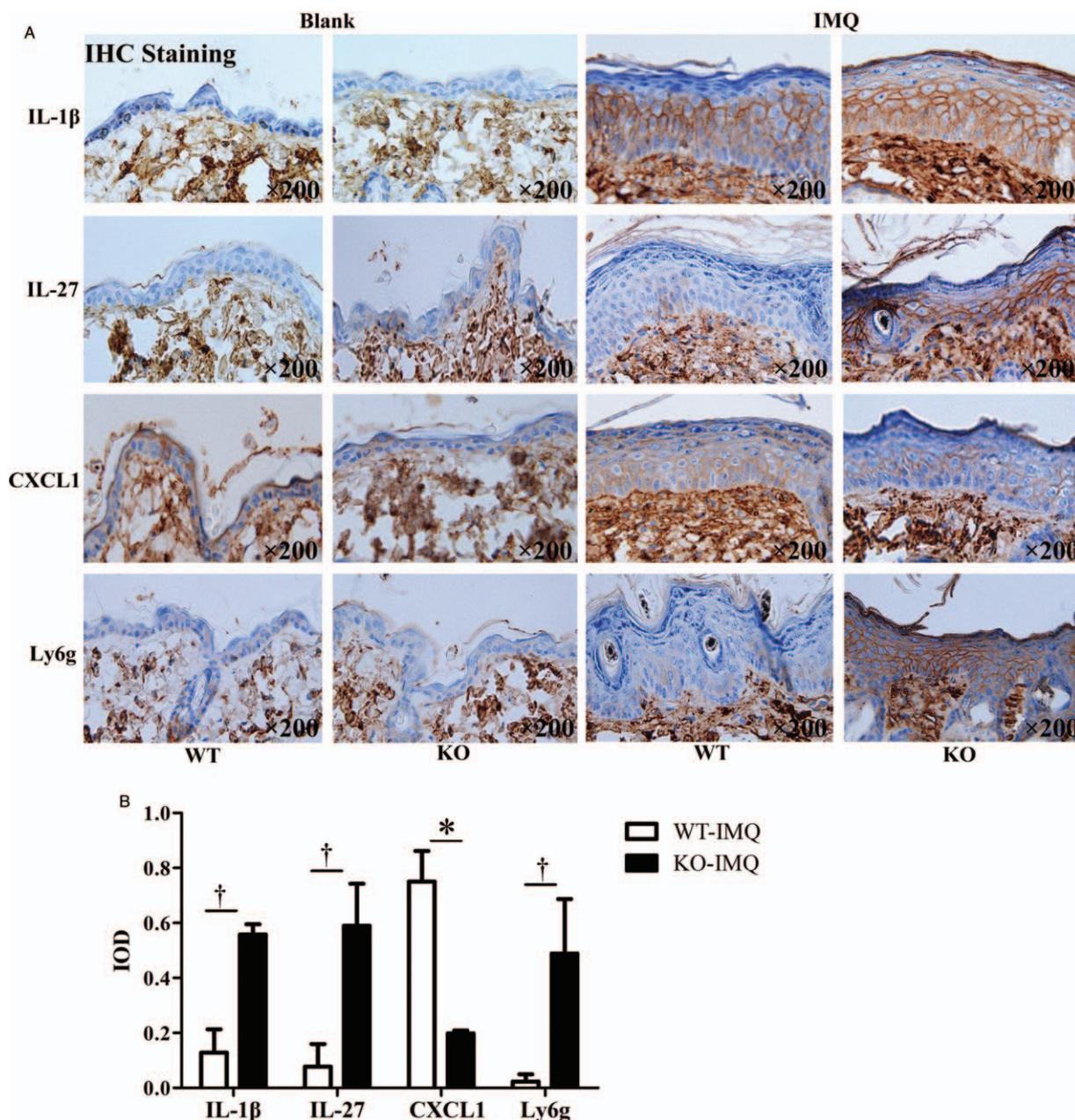


Figure 5: (A) Immunohistochemical staining (IHC, original magnification $\times 200$) of IL-1 β , IL-27, CXCL1, and Ly6g. (B) The expressions of IL-1 β , IL-27, and Ly6g were significantly increased in IMQ-treated KO compared to WT mice. Compared with IMQ-induced WT mice, CXCL1 displayed a lower expression level in KO mice. * $P < 0.05$, † $P < 0.01$, Blank: Control mice; IMQ: Imiquimod; KO: *IL-18* knockout mice; WT: Wild-type mice.

signaling pathway as that of IL-18. As a result, IL-18 appears to be related to IL-1 β in several processes.^[21] IL-1 β induces CD4⁺ and CD8⁺T cell activation and stimulates $\gamma\delta$ T cell transformation into Th17 cells. Except driving the Th17 response, IL-1 β promotes keratinocyte proliferation and production of chemokines involved with recruiting neutrophils.^[22] Here, we show that *IL-1 β* mRNA and IL-1 β protein were significantly increased in IMQ-induced *IL-18* KO compared to WT mice [Figures 4A and 5]. In *IL-18* deficient mice, skin lesion cells could enhance the production of IL-1 β to compensate for immunological dysfunctions due to its extreme similarity in structure and function to IL-18. The high levels of IL-1 β expression in *IL-18* KO mice might partly

result from IMQ stimulating keratinocytes to produce IL-1 β .^[14]

The results of our current study also demonstrate that the protective cytokines, *IL-4* and *IL-27*, were increased in IMQ-induced *IL-18* KO mice. Serum *IL-4* levels in active psoriatic patients are significantly lower than that of patients in stable stage.^[23] *IL-4* has anti-inflammatory properties, which can then downregulate IL-1 β , TNF α , IL-18, IL-23, and IL-17 production within many different cell types such as keratinocytes, monocytes, DC, and macrophages.^[24-26] Inflammatory cytokines can, in turn, inhibit *IL-4* gene expression.^[23] The results of our study indicate that *IL-18* might decrease the production of *IL-4*,

which may then inhibit the Th2 response. IL-27 reduces the production of IL-18 by robustly enhancing IL-18 binding protein (IL-18BP), which then inhibits the expression and activation of IL-18.^[27] It should be noted that there exist conflicting results regarding IL-27's role in the pathogenesis of psoriasis. IL-27 exerts anti-inflammatory effects by inhibiting Th17 cell differentiation, but also stimulates Th1 cell activation.^[28] It appears that IL-18 might reduce the expression of IL-27, however, the major role of IL-27 in psoriasis-like skin inflammation clearly requires further investigation.

CXCL1, an important neutrophil recruiting chemokine, was significantly decreased in *IL-18* KO compared to WT mice.^[14] A study involving oral keratinocytes showed that IL-1 network upregulates CXCL1.^[29] Our results indicate that IL-18 may promote epidermal cells to secrete CXCL1.

The IMQ-induced *IL-18* KO mice showed a milder degree of skin lesions and dermal cell infiltration compared with that observed in WT mice. The leukocyte infiltrate in psoriasis consists predominantly of T cells, and their infiltration precedes epidermal hyperplasia.^[30,31] As T cells are associated with the formation of psoriatic lesions,^[30] the milder phenotype observed in *IL-18* KO mice may be related to lower levels of T cell infiltration rather than that attributable to neutrophils. Larger areas in Munro microabscesses were obtained in *IL-18* KO *vs.* WT mice. In addition, Ly6g, a surface protein of neutrophils, showed higher expression levels in *IL-18* KO mice. These results indicate that neutrophils may be increased in *IL-18* KO mice. The role of neutrophils in psoriasis is not entirely understood. Although they appear to recruit T lymphocytes, they may not be directly related to severity of skin lesions.^[32] The mechanisms responsible for the lack of a significant phenotype effect and increased area of Munro microabscesses will require further investigation.

Results of our HE staining revealed that *IL-18* KO mice showed increased areas of scale formation as compared with WT mice. However, such differences were not apparent when gross observations were performed in these mice. It indicated that IL-18 might affect the adhesiveness of keratin, but there are few reports on IL-18 and scales in IMQ-induced psoriasis-like skin inflammation. The mechanisms involved in this process are unclear.

Our study represents an initial attempt at examining the functions of IL-18 protein in psoriasis. With the use of a conventional *IL-18* KO mouse, the *IL-18* gene will be knocked out in all cells. Such a condition would affect all immune-associated cells and organ functions related to IL-18, which may then result in a dysfunction of the IMQ-induced skin inflammation response. Therefore, a specific conditional gene knock-out mouse model might provide a more accurate model in which to assess the role of IL-18 in the development of psoriasis. While the analyses of sections resulting from HE staining and immunohistochemistry provide important information, their semi-quantitative parameters lack the rigor of methods that can provide more robust quantitative measures. Nevertheless,

we believe the data presented in this initial report on IL-18 and psoriasis provide a strong foundation for future work on the specific mechanisms of IL-18 in human psoriasis and for the development of new therapeutic approaches in the treatment of this condition. For future research, we will further explore the specific mechanism that IL-18 affect formation of scales and Munro microabscesses in IMQ-induced mouse model of psoriasis.

Funding

This work was supported by National Natural Science Foundation of China (Nos. 81673070, 81872538).

Conflicts of interest

None.

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How to cite this article: Niu XL, Huang Y, Gao YL, Sun YZ, Han Y, Chen HD, Gao XH, Qi RQ. Interleukin-18 exacerbates skin inflammation and affects microabscesses and scale formation in a mouse model of imiquimod-induced psoriasis. *Chin Med J* 2019;132:690–698. doi: 10.1097/CM9.0000000000000140