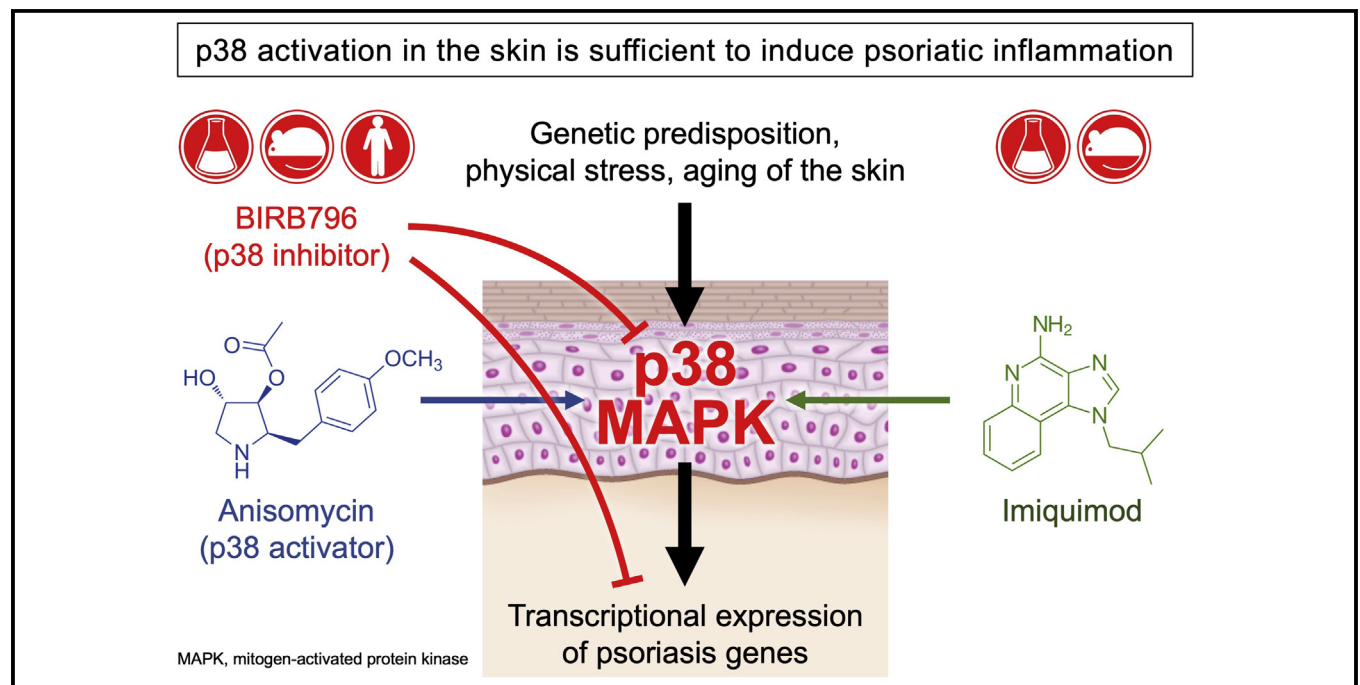


Cutaneous p38 mitogen-activated protein kinase activation triggers psoriatic dermatitis

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GRAPHICAL ABSTRACT



Background: Psoriasis is a chronic inflammatory skin disease characterized by IL-17-mediated immune responses. p38 is known to be highly activated in the psoriatic epidermis; however, whether p38 is involved in the development of psoriasis is unclear.

Objective: We sought to demonstrate that activation of p38 mitogen-activated protein kinase is sufficient to induce psoriatic inflammation in mice and that cutaneous p38 activities are the topical therapeutic targets for psoriasis.

Methods: A p38 activator, anisomycin, was applied daily to murine skin. Transcriptomic analyses were performed to evaluate the similarities of the skin responses to those in human psoriasis and the existing animal model. BIRB796, a small-molecule inhibitor targeting p38 activities, was applied to the murine psoriatic models topically or to human psoriatic skin specimens *ex vivo*.

Results: Topical treatment with anisomycin induced key signatures in psoriasis, such as epidermal thickening, neutrophil

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infiltration, and gene expression of *Il1a*, *Il1b*, *Il6*, *Il24*, *Cxcl1*, *Il23a*, and *Il17a*, in treated murine skin. These responses were fully abrogated by topical treatment with BIRB796, and were reduced in IL-17A-deficient mice. Transcriptomic analyses demonstrated the similarities of anisomycin-induced dermatitis to human psoriasis and imiquimod-induced murine psoriatic dermatitis. Furthermore, BIRB796 targeting of p38 activities reduced expression of psoriasis-related genes in both human keratinocytes stimulated with recombinant IL-17A *in vitro* and psoriatic skin specimens *ex vivo*.

Conclusion: Therefore our findings suggest that cutaneous p38 activation can be a key event in patients with psoriasis and a potential topical therapeutic target of a small molecule. (J Allergy Clin Immunol 2019;■■■:■■■-■■■.)

Key words: Psoriasis, p38 mitogen-activated protein kinase, anisomycin, IL-17

Psoriasis vulgaris is a common chronic inflammatory skin disease that affects 2% to 3% of the global population and impairs the quality of life of affected subjects. The condition is characterized by plaques, epidermal hyperplasia, and skin infiltration of leukocytes, including neutrophils.^{1,2} T_H17 has recently been identified as the primary T-cell subset involved in the pathogenesis of psoriasis.^{3,4} Inflammatory myeloid dendritic cells (DCs) release IL-23 and activate T_H17 cells to produce IL-17 and IL-22.¹ These T_H17 cytokines contribute to epidermal abnormalities in keratinocyte proliferation and differentiation. In addition, T_H17 cytokines promote keratinocytes to produce chemokines, such as CXCL1, which recruits neutrophils, and CCL20, which recruits T_H17, respectively, to psoriatic lesions, establishing a positive feedback loop of IL-17-mediated inflammation.⁵

Psoriasis is thought to develop on a basis of genetic susceptibility, which is associated with signaling disorders and production of immune mediators, resulting in defective immune homeostasis in the skin.¹ Recent analyses of gene mutations in patients with familial-type psoriasis and single nucleotide polymorphisms in patients with psoriasis have suggested that the significant contribution of innate immune response of the skin in disease susceptibility.^{1,6-8} Indeed, many psoriasis-related gene products originate in the epidermis.⁹ However, a specific signaling pathway that is preferentially activated in predisposed subjects and can induce IL-17-mediated psoriatic inflammation remains elusive.^{1,2,10,11}

Mitogen-activated protein kinases (MAPKs) are signaling components that play important roles in converting and transferring extracellular stimuli, such as cell stresses, into a wide range of cellular responses. The role of the IL-17 pathway is also considered to depend in part on activation of p38 MAPK.¹² Activation of p38 MAPK in the epidermis has also been suggested to be involved in the pathogenesis of psoriatic inflammation based on several observations. First, p38 is highly activated in the epidermis in patients with human lesional psoriasis.^{13,14} Second, retinoids, which are often used for the treatment of psoriasis, are known to suppress production of several keratinocyte mediators, such as matrix metalloproteinases, by possibly regulating p38 activation.¹⁵ Third, phosphorylation of signal transducer and activator of transcription 3 activation, which has a definitive role in psoriatic inflammation in the epidermis,¹⁶ is induced in part by p38.^{17,18} Lastly, p38 induces IL-24 transcription,¹⁹ which

Abbreviations used

CARD14:	Caspase recruitment domain-containing protein 14
DC:	Dendritic cell
DEG:	Differentially expressed gene
DETC:	Dendritic epidermal T cell
DMSO:	Dimethyl sulfoxide
JNK:	c-Jun N-terminal kinase
MAPK:	Mitogen-activated protein kinase
NF-κB:	Nuclear factor κB
NHEK:	Normal human epidermal keratinocyte

is induced in lesion of patients with psoriasis²⁰ and precedes spontaneous development of psoriasis-like dermatitis in animals lacking nuclear factor κB (NF-κB) in the epidermis.^{21,22} Thus it can be postulated that predominant activation of p38 in the skin is the key event in development of psoriatic lesions.

In the present study we found that daily topical treatment with anisomycin, a p38 activator, on mouse ear skin rapidly induces psoriatic dermatitis in a dose-dependent and IL-17A-dependent manner. Transcriptomic analyses also demonstrate the similarities of anisomycin-induced dermatitis to human psoriasis. Furthermore, p38 inhibitors have been shown to reduce expression levels of inflammatory cytokines in human psoriatic lesions *ex vivo*. These data suggest that breakdown of skin homeostasis through p38 activation is sufficient for development of psoriasis.

METHODS

Mice

Eight- to 12-week-old female mice were used in this study. All animals used were of the C57BL/6 genetic background. IL-17A-deficient mice and keratinocyte-specific p65 and c-Rel double-knockout mice were reported previously.^{21,23,24} All animals were maintained under specific pathogen-free conditions in the Institute of Laboratory Animals at Kyoto University Graduate School of Medicine. Experimental procedures were approved by the ethics committee for animal experiments and performed according to the Guidelines for Animal Experiments of Kyoto University. Sample sizes were determined based on previous experience. For each experiment, sample size reflects the number of independent biological replicates and is provided in the figure legend. Further details of animal experiments are provided in the [Methods](#) section in this article's Online Repository at www.jacionline.org.

Anisomycin and imiquimod treatment

Anisomycin (Wako Pure Chemical Industries, Osaka, Japan) solution was applied to the inner and outer sides of a murine ear once daily. The concentration of anisomycin was 2 mg/mL in 99.5% ethanol unless otherwise indicated because of the development of moderate levels of inflammation, and the dose was 10 μL per side of the ear. BIRB796 (Selleck Chemicals, Houston, Tex) or JNK-IN-7 (MedChem Express, Monmouth Junction, NJ) solution in ethanol was mixed with anisomycin solution to a 5 mg/mL concentration and applied to a murine ear. For the imiquimod model, 10 mg of imiquimod-containing cream (Aldara; Beselna Cream 5%; Mochida Pharmaceuticals, Tokyo, Japan) was applied to the inner and outer sides of a murine ear once daily.

Cell culture

Murine epidermis was harvested and subjected to keratinocyte preparation performed by using the following methods. In general, full-thickness skin was

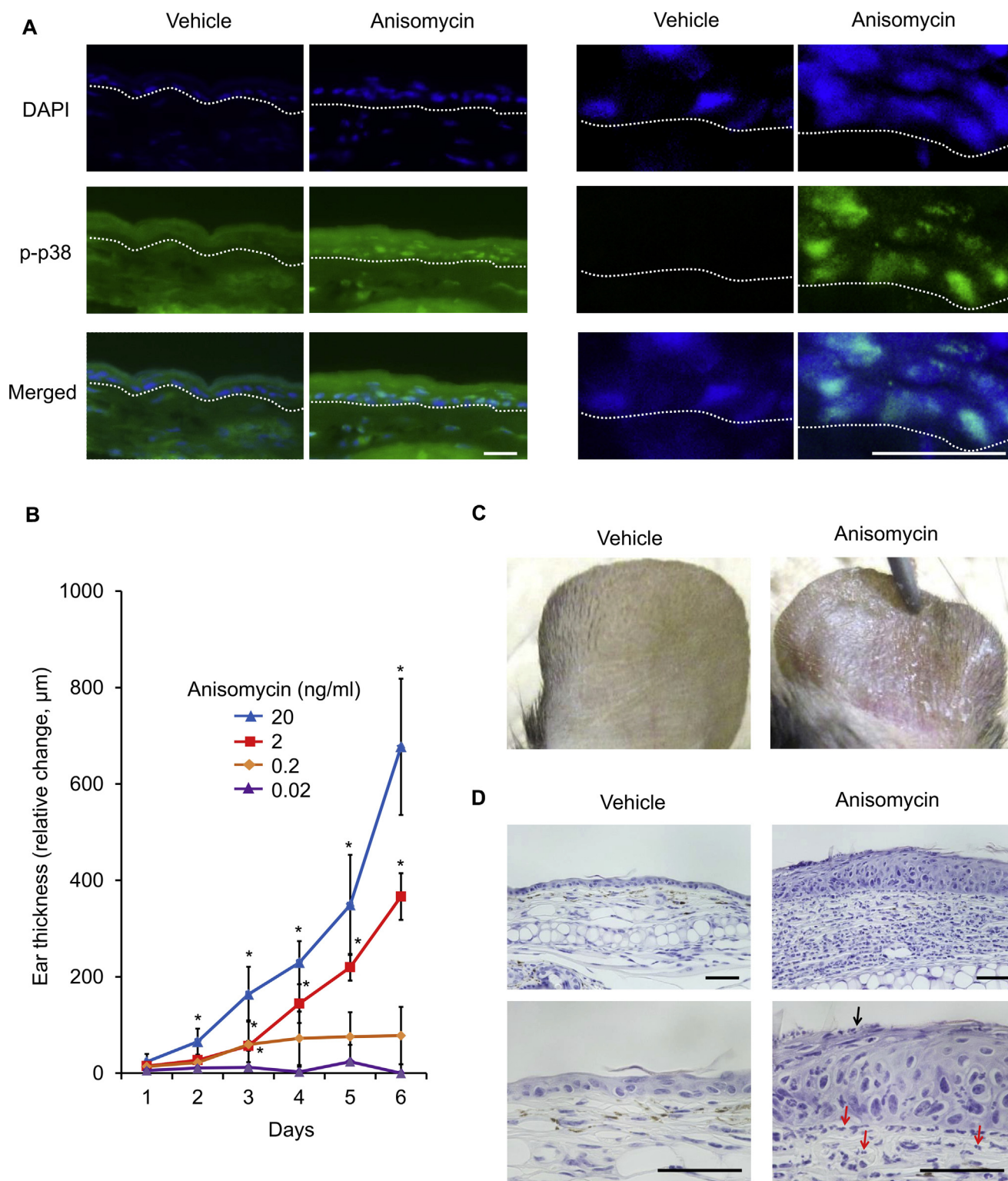


FIG 1. Anisomycin-induced skin inflammation in mice phenotypically resembles human psoriasis. **A**, Immunofluorescence staining of mouse ear skin topically treated with anisomycin for phosphorylated p38 (*p-p38*; green) and nuclei (blue). *Dashed lines* demarcate the dermoepidermal junction. *Scale bars* = 20 μ m. *DAPI*, 4'-6-Diamidino-2-phenylindole dihydrochloride. **B**, Time course of changes in ear thickness ($n = 3$ per group). Thickness was measured daily before treatment. $*P < .05$. **C**, Representative gross appearance of ears treated with or without daily anisomycin for 5 consecutive days. **D**, Histology of ear sections stimulated with or without 2 mg/mL anisomycin harvested on day 5. The *black arrow* shows microabscesses, and *red arrows* show neutrophils in the dermis (hematoxylin and eosin staining). *Scale bars* = 50 μ m.

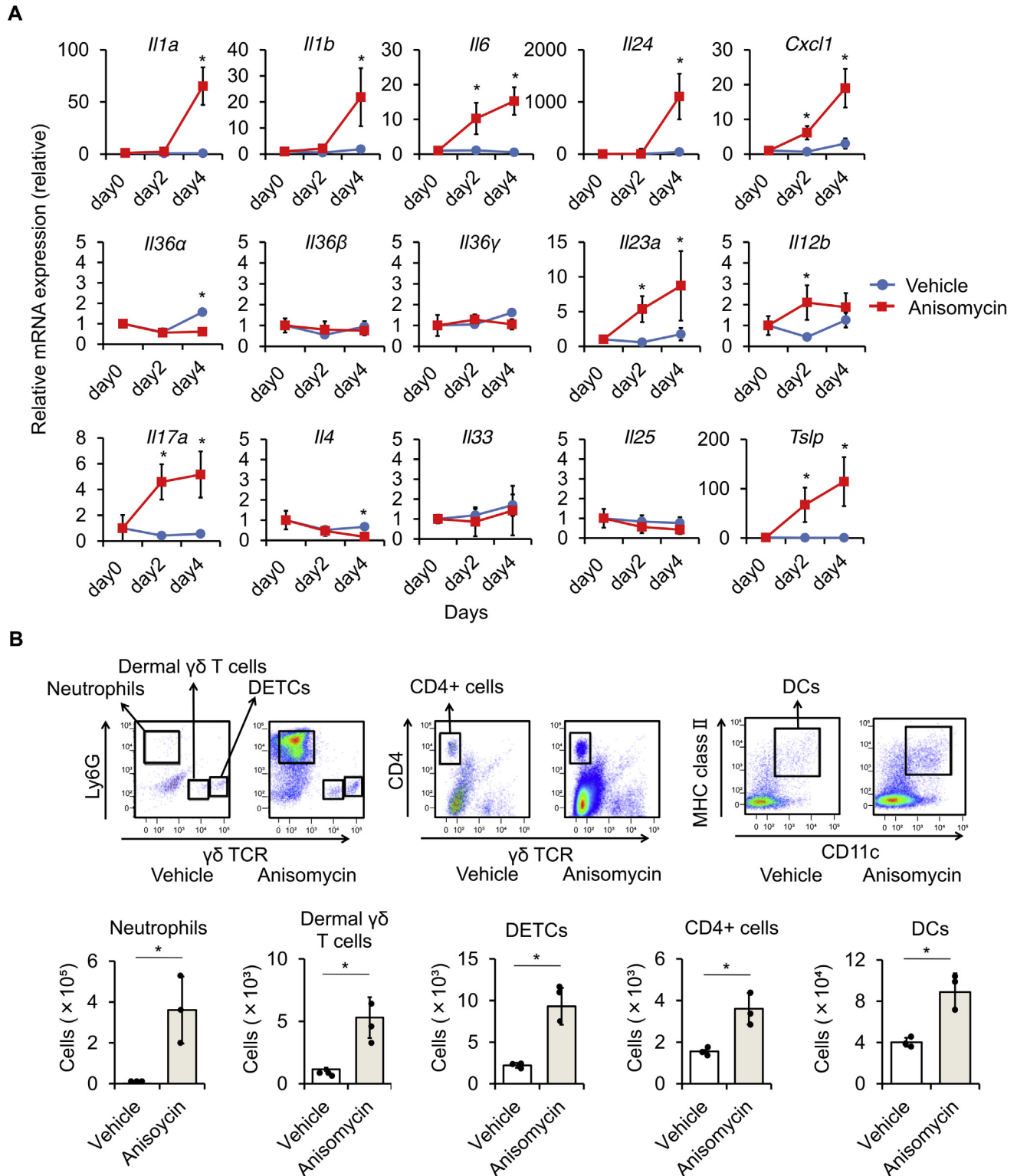


FIG 2. Anisomycin treatment induced psoriatic immune responses. **A**, Quantitative RT-PCR analysis of mRNA levels in murine ear skin treated with or without anisomycin on days 0, 2, and 4 ($n = 4$). Results were normalized to glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) expression and are shown as means $\pm$ SDs. * $P < .05$. **B**, Flow cytometric analyses of CD45⁺, CD4⁺, and Ly6G⁺ neutrophils; CD45⁺, CD4⁺, and $\gamma\delta$ T-cell receptor (TCR) middle⁺ $\gamma\delta$ T cells that are $\gamma\delta$ TCR positive at a level in the middle of a scale of $\gamma\delta$ TCR; CD45⁺, CD4⁺, and $\gamma\delta$ T-cell receptor-high⁺ DETCs; CD45⁺, CD4⁺, and $\gamma\delta$ TCR⁺ cells; and CD45⁺, MHC class II⁺, and CD11c⁺ DCs from ears of individual mice after 5 days of anisomycin treatment ($n = 3$). Results are expressed as means $\pm$ SDs. * $P < .05$.

obtained from newborn mice. Skin samples were floated and incubated in Dispase II (1 U/mL; Roche, Mannheim, Germany) overnight at 4°C. The epidermis was then separated from the dermis, cut into pieces, and incubated in 0.05% trypsin for 5 minutes. Cells were cultured in low-calcium medium (0.05 mmol/L Ca^{2+}) using Eagle minimum essential medium (Lonza, Basel, Switzerland) with chelated FCS. Cells were seeded on 12-well plates at a density of 3×10^5 cells per well for RNA extraction. For cell-signaling experiments, cells were serum starved for 24 hours before stimulation. Anisomycin (50 ng/mL) and BIRB796 (50 ng/mL) were dissolved in dimethyl sulfoxide (DMSO) and used for cell stimulation. Normal human epidermal keratinocytes (NHEKs; Kurabo, Osaka, Japan) were inoculated at a concentration of 2500 cells/cm² in culture medium, HuMedia-KG2 (Kurabo) supplemented with insulin (10 $\mu\text{g/mL}$), human epidermal growth factor (0.1 ng/mL), hydrocortisone (0.5 $\mu\text{g/mL}$), bovine pituitary extract (0.4% vol/vol), gentamicin (50 $\mu\text{g/mL}$), and amphotericin B (50 ng/mL). The culture was maintained at 37°C in a 95% O_2 /5% CO_2 humidified chamber. Anisomycin (50 ng/mL) and BIRB796 (50 ng/mL) were dissolved in DMSO and used for cell stimulation. rIL-17A (200 ng/mL; R&D Systems, Minneapolis, Minn) was used to stimulate primary keratinocytes, and BIRB796 (500 ng/mL) in DMSO was used as an inhibitor. Further details of *in vitro* experiments, including microarray analysis, are provided in the [Methods](#) section in this article's Online Repository, which cites [Tables E1](#) and [E2](#).

Human samples

We obtained biopsy specimens of psoriatic lesions from 10 patients (8 men and 2 women) aged 22 to 77 years (average, 49.9 years) at Kyoto University Hospital after obtaining informed consent. Two adjacent biopsy specimens were taken from each patient. All specimens were cut at the upper dermis, and the epidermal side was used for the experiment. As a medium, HuMedia-KB2 (Kurabo) containing insulin, human epidermal growth factor, bovine pituitary extract, and gentamicin/amphotericin B was used, according to the manufacturer's protocol. One specimen was cultured with vehicle (DMSO), and the adjacent one was cultured with BIRB796 (500 ng/mL) for 12 hours.

Statistics

Unless otherwise indicated, data are presented as means $\pm$ SDs, and each data point is representative of 3 independent experiments. Two-tailed Student *t* tests were performed with Excel software (Microsoft, Redmond, Wash) to assess statistical significance unless otherwise indicated. The Wilcoxon signed-rank test was performed with Stata/SE software (version 14.2; StataCorp, College Station, Tex) for analysis of nonparametric paired data from studies using human tissue specimens.

RESULTS

Topical treatment with a p38 MAPK activator induced psoriatic dermatitis *in vivo*

We hypothesized that activation of p38 MAPK in keratinocytes is involved in development of psoriatic inflammation through production of proinflammatory mediators. First, we confirmed that anisomycin treatment induced phosphorylation of p38 by using murine keratinocytes (see [Fig E1, A](#), in this article's Online Repository at www.jacionline.org). We then found that anisomycin treatment induced transcriptional expression of several psoriasis-related genes, including those encoding IL-1 α , IL-1 β , IL-6, and CXCL1, but not expression of T_H2-mediated atopic dermatitis-related genes encoding IL-33 and IL-25 (see [Fig E1, B](#)). These findings were also demonstrated in NHEKs (see [Fig E1, C](#)).

We next anticipated that continuous activation of p38 in the skin might induce development of psoriatic dermatitis *in vivo*.

Topical anisomycin treatment of the skin *in vivo* induced phosphorylation of p38 predominantly in epidermal cells of mouse ears ([Fig 1, A](#)). Ear thickness showed a dose-dependent increase on daily topical anisomycin application to mouse ears ([Fig 1, B](#)). Ears treated with anisomycin exhibited scaly erythema mimicking human psoriatic lesions ([Fig 1, C](#)). Histologic sections of anisomycin-treated sites showed epidermal thickening, infiltration of lymphocytes and neutrophils into the dermis, and epidermal accumulation of neutrophils forming microabscesses, all similar to the features of human psoriasis ([Fig 1, D](#)).

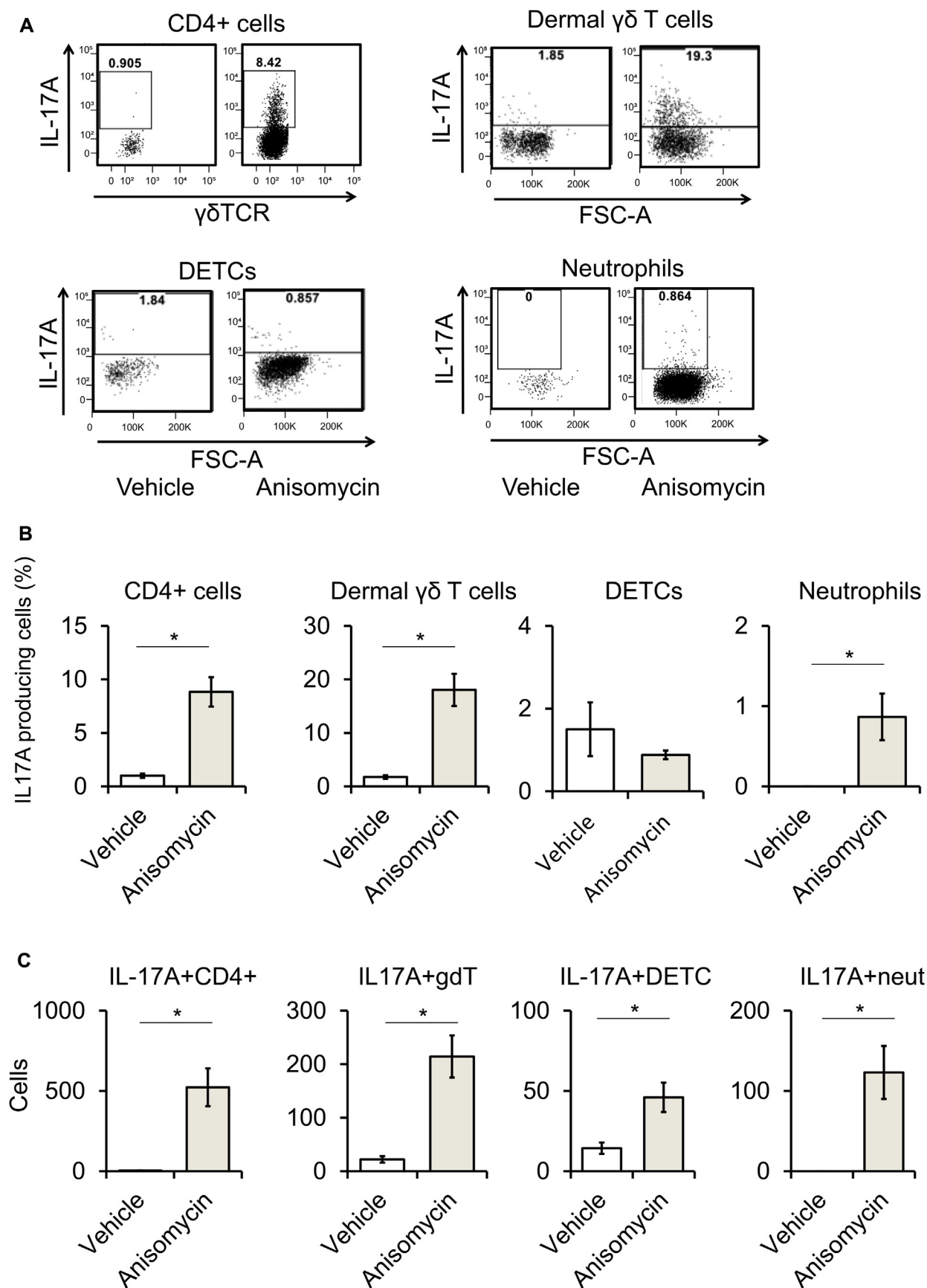
To evaluate whether anisomycin-induced dermatitis mimics the psoriasis immune response, we conducted RT-PCR analysis of whole ear skin with or without anisomycin treatment. Expression levels of several psoriasis-related genes, including *Il1a*, *Il1b*, *Il6*, *Il24*, *Cxcl1*, *Il23a*, and *Il17A*, were upregulated in the anisomycin-treated whole ear, whereas gene expression levels of *Il4*, *Il33*, and *Il25*, which are involved in T_H2-type immune responses, were not ([Fig 2, A](#)). Thymic stromal lymphopoietin, expression of which is increased in the lesional skin of patients with psoriasis,²⁵ was markedly induced by anisomycin treatment. On the other hand, expression of IL-36 α , IL-36 β , and IL-36 γ , which are known to be involved in the pathogenesis of psoriasis,²⁶ was not induced by anisomycin treatment.

To examine cells infiltrating daily to ears treated with anisomycin, we conducted flow cytometric analyses. Numbers of neutrophils, dermal $\gamma\delta$ T cells, dendritic epidermal T cells (DETCs), CD4⁺ cells, and DCs in skin treated with anisomycin were greater than those in skin treated with the vehicle control ([Fig 2, B](#)). It has been reported that IL-17 is produced by CD4⁺ cells, $\gamma\delta$ T cells, and neutrophils in patients with psoriatic inflammation.^{27,28} In skin lesions the percentages of IL-17A-producing cells among CD4⁺ cells, $\gamma\delta$ T cells, and neutrophils ([Fig 3, A and B](#)) and the numbers of IL-17A-producing CD4⁺ cells, $\gamma\delta$ T cells, DETCs, and neutrophils in the anisomycin-treated group were greater than those in the vehicle control group ([Fig 3, C](#)).

These results demonstrate that activation of p38 in the skin is sufficient to induce IL-17A-producing cells and psoriatic dermatitis.

Topical treatment with a p38 inhibitor suppressed development of anisomycin-induced dermatitis

Anisomycin activates c-Jun N-terminal kinase (JNK), as well as p38. To confirm whether anisomycin-induced dermatitis especially depends on p38 activation, we used the p38-specific inhibitor BIRB796. We first confirmed that BIRB796 treatment completely abolished the anisomycin-induced phosphorylation of p38 and the transcriptional expression of psoriasis-related genes in murine keratinocytes *in vitro* (see [Fig E1, A](#)). Daily topical treatment with BIRB796 attenuated the development of anisomycin-induced psoriatic dermatitis in mice ([Fig 4, A-C](#)). The increased ear thickness, scaly erythema, and histologic changes in mice induced by anisomycin were attenuated by BIRB796 treatment, whereas topical application of the JNK inhibitor JNK-IN-7 did not suppress anisomycin-induced ear swelling (see [Fig E2](#) in this article's Online Repository at www.jacionline.org). These results suggest that development of psoriatic dermatitis in this animal model is due to continuous activation of cutaneous p38.



Anisomycin-induced psoriatic dermatitis depends on IL-17A

Predominant production of IL-17A in anisomycin-treated skin (Fig 3) suggests that cutaneous activation of p38 gives rise to accumulation and activation of IL-17A-producing cells in the skin, which are critical in the development of psoriatic dermatitis.

To assess whether IL-17A plays an important role in this animal model, we topically treated IL-17A-deficient mice and wild-type mice with anisomycin, comparing the effect on the severity of their skin lesions. Anisomycin-induced psoriatic dermatitis (erythema and thickening of the ears) and histologic changes (epidermal thickening) were much more attenuated in IL-17A-deficient mice than in wild-type mice (Fig 4, D-F). In addition, epidermal accumulation of neutrophils (microabscesses) was not observed in anisomycin-treated IL-17A-deficient mice (Fig 4, F). Collectively, these results suggest that development of anisomycin-induced psoriatic dermatitis is dependent on IL-17A because IL-17A has a crucial role in development of human psoriasis and its animal models.²⁹

Gene expression profile of anisomycin-induced dermatitis simulates that of human psoriasis

Murine psoriatic dermatitis induced by topical treatment with imiquimod, a Toll-like receptor 7 agonist, has been widely used for psoriasis research.³⁰⁻³² This animal model for psoriasis shares IL-17 activation and its dependency with anisomycin-induced dermatitis.

We next compared the transcriptomic signature of the anisomycin-induced dermatitis model or those of imiquimod-induced dermatitis to human psoriasis to further confirm their similarities and characterize these animal models. The heat map showed that genes upregulated in patients with anisomycin-induced dermatitis are also largely upregulated in imiquimod-induced dermatitis and *vice versa*, but the expression patterns were in part different between these 2 models (Fig 5, A). Approximately two thirds of the genes upregulated in patients with imiquimod-induced dermatitis were also induced in patients with anisomycin-induced dermatitis (Fig 5, B). The top representative differentially expressed genes, including genes upregulated and downregulated only in patients with anisomycin-induced dermatitis or in those with imiquimod-induced dermatitis, in addition to genes upregulated in both, are summarized in Table I. Many key psoriasis-related genes, such as *Krt6b* encoding keratin 6b, *Il1b*, and *Saa3* encoding serum amyloid a3, were upregulated in both animal models. Some genes with possible roles in psoriasis, such as *Il6* and *Cxcr4*, were significantly upregulated in patients with anisomycin-induced dermatitis, whereas others, such as *Ccl20*, were predominantly upregulated in patients with imiquimod-induced dermatitis (Fig 5, C). Some genes predominantly expressed in the anisomycin model, such as *Cxcl2* and *Ccl3*, showed a signature

of neutrophil recruitment, whereas gene expression levels of some antimicrobial peptides and proteins, such as *Defb3*, *S100a8*, and *S100a9*, were dominant in the imiquimod model. As for IL-20 receptor ligands, gene expression levels of *Il24* were dominant in the anisomycin model, whereas those of *Il19* encoding IL-19 were dominant in the imiquimod model.

Pathway analysis with human psoriasis transcriptomes^{33,34} demonstrated that both animal models exhibited many similarities to human psoriasis in gene expression profiles, particularly in the IL-17 and TNF-associated cytokine pathways (Fig 5, D).^{1,35,36}

These results further characterize the anisomycin model and differentiate it from the imiquimod model, suggesting that these animal models could be advantageous and complementary for investigating the pathogenesis of psoriasis.

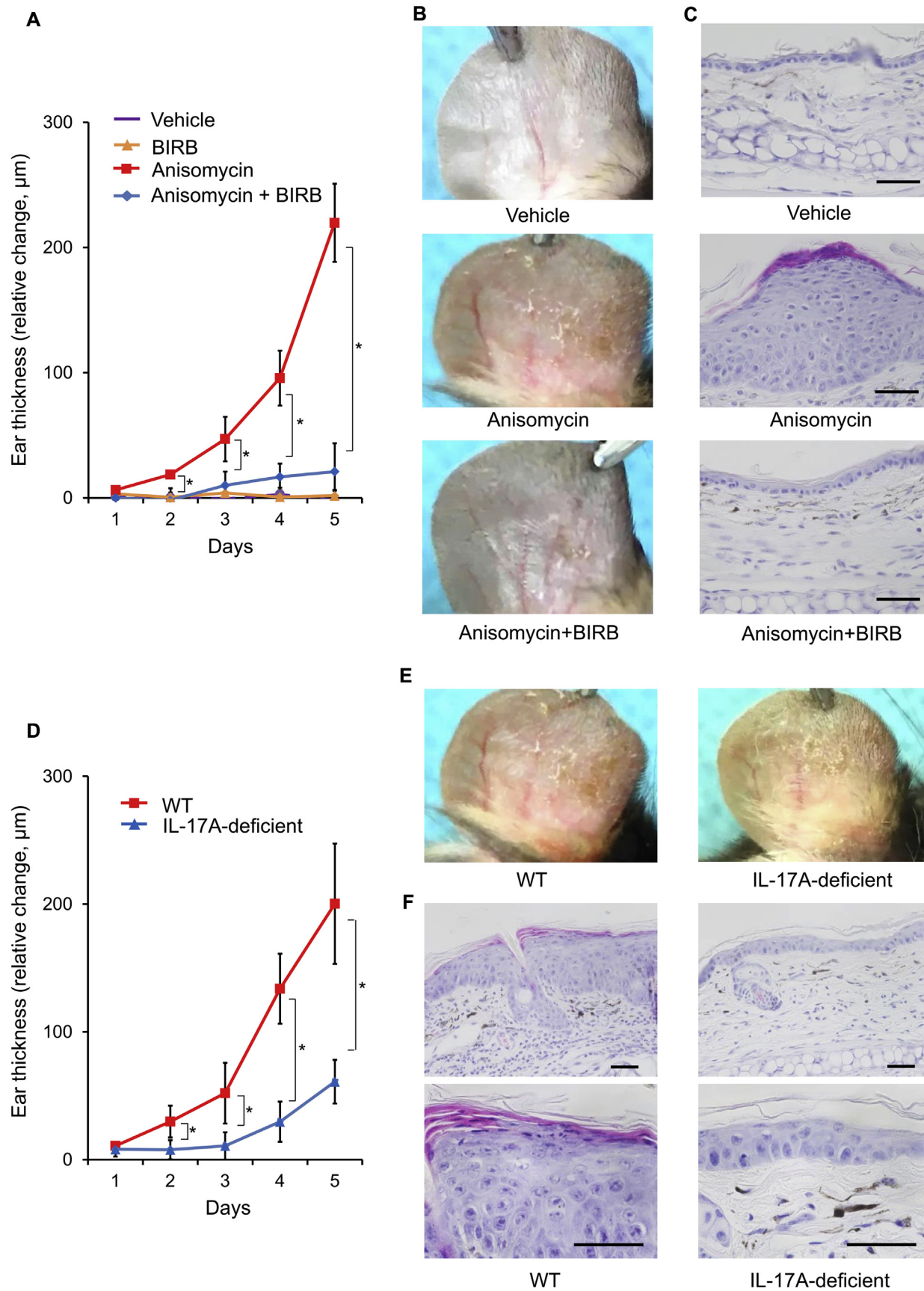
Topical treatment with a p38 inhibitor suppressed development of imiquimod-induced dermatitis

Mice lacking p65 and c-Rel specifically in the epidermis have spontaneous psoriatic dermatitis,²¹ and immunofluorescence studies detected phosphorylation of p38 in the epidermis (see Fig E3, A, in this article's Online Repository at www.jacionline.org). Immunofluorescence studies also demonstrated phosphorylation of p38 in epidermis topically treated with imiquimod (see Fig E3, B). BIRB796 treatment attenuated erythema and scaling of the ears induced by imiquimod application compared with vehicle treatment (see Fig E3, C). The ear swelling and epidermal thickening of imiquimod-induced dermatitis were partially suppressed by BIRB796 (see Fig E3, D-F). These results suggest that the involvement of cutaneous p38 activation in the development of IL-17-mediated dermatitis is shared among these animal models.

BIRB796 suppressed psoriasis-related gene expression levels of NHEKs and lesional skin of patients with psoriasis

We were tempted to speculate that topical treatment with the p38 inhibitor BIRB796 can attenuate psoriatic inflammation in human subjects, although a clinical study with BIRB796 systemic treatment did not show definitive efficacy in patients with psoriasis.³³ We examined whether BIRB796 suppresses inflammatory responses in keratinocytes stimulated with rIL-17A because the positive feedback loop of IL-17 directly activates keratinocytes and promotes the persistence of psoriatic plaques,³⁴ and the IL-17 pathway is considered to depend in part on p38 activation.¹² Quantitative RT-PCR analysis revealed that BIRB796 suppressed the IL-17A-induced expression of several psoriasis-related genes, including *IL1A*, *IL1B*, *IL6*, *IL24*, and *CXCL1*, in NHEKs (Fig 6, A). Furthermore, we tested whether BIRB796 suppresses psoriasis-related gene expression

FIG 3. Anisomycin treatment induced IL-17A-producing cells in the skin. IL-17A⁺ cells from ears of individual mice after 5 days of anisomycin treatment (n = 3) are shown. **A**, Dot plots show CD45⁺, CD4⁺, γδ T-cell receptor (TCR)⁺, and IL-17A⁺ T cells; CD45⁺, CD4⁺, γδ TCR middle⁺, and IL-17A⁺ dermal γδ T cells; CD45⁺, CD4⁺, γδ TCR high⁺, and IL-17A⁺ DETCs; and CD45⁺, CD4⁺, Ly6G⁺, and IL-17A⁺ neutrophils. Results are expressed as means ± SDs. *P < .05. FSC, Forward scatter. **B**, Percentage of IL-17A-producing cells among each cell subset. **C**, Number of CD45⁺, CD4⁺, γδ TCR⁺, and IL-17A⁺ T cells; CD45⁺, CD4⁺, γδ TCR middle⁺, and IL-17A⁺ dermal γδ T cells; CD45⁺, CD4⁺, γδ TCR high⁺, and IL-17A⁺ DETCs; and CD45⁺, CD4⁺, Ly6G⁺, and IL-17A⁺ neutrophils.



levels in lesional skin from patients with psoriasis *ex vivo*. Despite the limited number of cases, BIRB796-treated psoriasis specimens tended to show lower expression levels of psoriasis-related inflammatory genes, such as *IL1B*, *IL6*, *IL23A*, and *IL17A*, than control specimens (Fig 6, B and C), whereas expression levels of some psoriasis-related genes, including antimicrobial peptide genes, were comparable between BIRB796-treated specimens and their paired controls (see Fig E4 in this article's Online Repository at www.jacionline.org). These results suggest that the p38 signaling pathways in psoriatic skin might be a potential therapeutic target and that topical p38 inhibitors might be effective in the treatment of psoriasis.

DISCUSSION

We showed that topical activation of p38 MAPK in the skin using daily topical anisomycin treatment is sufficient to induce IL-17A-dependent psoriatic dermatitis in mice. Moreover, treatment with a p38-specific inhibitor attenuated expression of inflammatory mediators in human keratinocytes stimulated with IL-17A *in vitro* or psoriatic lesions *ex vivo*. Therefore our findings suggest that activation of p38 MAPK in the skin is involved in IL-17A-dependent pathogenesis of psoriasis.

We propose that the anisomycin-induced dermatitis model might more directly represent the trigger for development of human psoriasis than existing psoriasis animal models. An assumed schematic diagram of imiquimod and the anisomycin model is presented in Fig 6, D. Imiquimod-induced dermatitis is more complicated than formerly expected: because of evident vehicle activities, it is not just attributed to the simple activation of Toll-like receptor signaling pathways that might simulate signaling events in psoriasis.²³ Another psoriasis animal model induced by means of subcutaneous injection with IL-23 is not appropriate to investigate the upstream events of IL-23 production in patients with psoriatic dermatitis.³⁷ Development of anisomycin-induced dermatitis was largely dependent on activation of p38 MAPK and was sufficient for disease development. Therefore our animal model is expected to closely simulate the cutaneous signaling abnormalities in patients with psoriasis and to provide an additional tool to investigate psoriasis pathogenesis. Especially, this model might contribute to further investigation of the roles of neutrophils and IL-24 in patients with psoriasis because neutrophil infiltration and IL-24 expression are dominant in this new model.

There are some limitations of this animal model. First, gene expression of some antimicrobial peptides and proteins, such as *Defb3*, *S100a8*, and *S100a9*, in lesional skin were more dominant in an imiquimod model than in this model (Fig 5, C).

Second, expression levels of some psoriasis-related genes, such as *KRT16*, *S100A9*, and *KLK6*, in lesional specimens from

patients with psoriasis did not show any marked reduction by using *ex vivo* treatment with BIRB796 in our system (see Fig E4). This result raises the concern that topical treatment of the p38 inhibitor might protect against further development of psoriatic skin but might not have a prompt or strong effect on already developed psoriatic skin. However, this limitation can be attributed to the evaluation system *ex vivo* because it might not be useful for some psoriasis-related genes to detect the changes of expression levels through overnight culture of skin specimens.

Third, IL-36 cytokines were not induced by anisomycin treatment (Fig 2, A).

Fourth, kinases other than p38 might be involved in pathogenesis of anisomycin-induced dermatitis because anisomycin activates p38 and JNK and BIRB796 could also affect JNK activities. To this end, we examined whether a JNK inhibitor modulates anisomycin-induced dermatitis, but the JNK inhibitor did not suppress it (see Fig E2).

We found that physical stress to the skin rapidly induced transient activation of p38 in the epidermis (see Fig E5 in this article's Online Repository at www.jacionline.org), but physical stress to the skin is not enough to trigger psoriatic inflammation in a healthy population. In patients with psoriasis, excessive responses of the cutaneous tissue to external stresses are considered important drivers despite the disease heterogeneity and multiple pathogeneses proposed.^{38,39} Thus our animal model suggests that abnormal p38 activation induced by external stimuli and continuous activation of the IL-17 pathway in endogenously disordered skin potentially result in development and propagation of psoriasis and that dysregulated activation of the upstream molecules that cause p38 activation in the skin might be involved in the pathogenesis. This is consistent with the epidemiologic evidence that psoriasis develops during middle age and that the p38 pathway activation in the skin of aged subjects is more inducible than that of young subjects.⁴⁰

Clinical and experimental evidence supports the important role of p38 activation in the skin for development of psoriasis. Recently, gain-of-function mutations in caspase recruitment domain-containing protein 14 (*CARD14*) have been reported in patients with familial-type psoriasis.⁷ *CARD14* is an epidermal activator of the p38 and JNK MAPK pathways, as well as NF- κ B pathways.⁴¹ In addition, loss-of-function mutations in *IL36RN*, which encodes IL-36 receptor antagonist, were described in families with severe pustular psoriasis,^{6,42} which is considered to be related to the uncontrolled activation of IL-36 receptor signaling pathways, such as p38 MAPK and NF- κ B pathways in keratinocytes.⁴³⁻⁴⁵ Moreover, mice lacking TNF receptor-associated factor 6 in keratinocytes do not have imiquimod-induced dermatitis, suggesting that the p38 MAPK and NF- κ B pathways in the epidermis are critical because TNF receptor-associated factor 6 is a key upstream molecule of these

FIG 4. Anisomycin-induced dermatitis was driven in a p38 activity- and IL-17A-dependent manner. **A**, Time course of changes in ear thickness ($n = 4$ per group). Thickness was measured daily before treatment. Values are shown as means $\pm$ SDs. $*P < .05$. **B**, Representative gross appearance of ears treated for 5 consecutive days. **C**, Histology of ear sections harvested on day 5 (hematoxylin and eosin staining). Scale bars = 50 μ m. **D**, Time course of changes in ear thickness ($n = 4$ per group). Thickness was measured daily before treatment. Wild-type (WT) and IL-17A-deficient (IL-17A KO) mice were treated with daily anisomycin for 5 consecutive days. Values are shown as means $\pm$ SDs. $*P < .05$. **E**, Representative gross appearance of ears treated with daily anisomycin for 5 consecutive days. **F**, Histology of the ear sections harvested on day 5 (hematoxylin and eosin staining). Scale bars = 50 μ m.

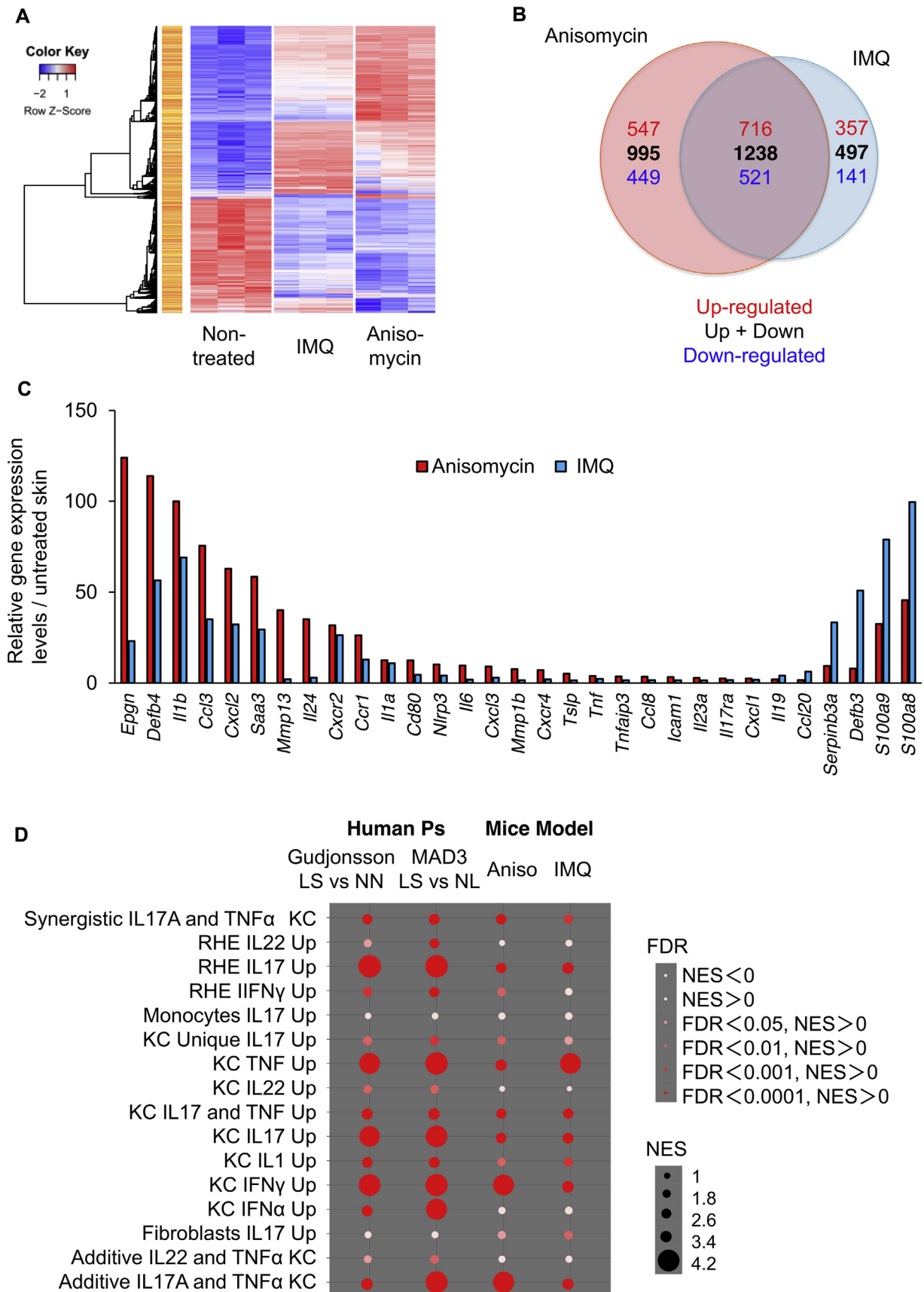


TABLE I. Top representative differentially expressed genes in the setting of anisomycin- and imiquimod-induced dermatitis

Anisomycin				Imiquimod				Anisomycin and imiquimod												
Upregulated		Downregulated		Upregulated		Downregulated		Upregulated						Downregulated						
Gene	FCH	Gene	FCH	Gene	FCH	Gene	FCH	Gene sort by	FCH-	FCH-	Gene sort by	FCH-	FCH-	Gene sort by	FCH-	FCH-	Gene sort by	FCH-	FCH-	
Gene	FCH	Gene	FCH	Gene	FCH	Gene	FCH	anisosmynin	anisosmynin	imiquimod	imiquimod	anisosmynin	imiquimod	anisosmynin	imiquimod	anisosmynin	imiquimod	imiquimod	anisosmynin	imiquimod
GPRC5A	34.65	KRTAP7-1	-8.19	ALOX8	29.85	CAR6	-4.05	KRT6B	233.65	211.57	KRT6B	233.65	211.57	KRT24	-22.06	-8.66	SERPI	-16.70	NA3B	
TMPRSS11B	33.50	2300005	-6.01	LPO	22.05	DES	-2.81	LCE3E	212.99	127.68	LCE3E	212.99	127.68	SERPI	-16.70	-12.32	KRT77	-4.46	NA3B	
ADM	15.35	ALDH3A1	-4.76	AOX4	15.07	MYOT	-2.80	EPGN	124.01	23.08	KRT16	122.55	102.87	FLG2	-10.54	-6.98	KRT24	-22.06		
INHBA	11.31	CYP2B19	-4.58	UOX	11.75	EFHD1	-2.79	KRT16	122.55	102.87	S100A8	45.57	99.59	ALOX12E	-9.00	-5.74	CYP2G1	-5.44		
LAMC2	10.47	ADH7	-4.34	SLC13A3	9.08	AMPD1	-2.76	DEFB4	113.92	56.46	S100A9	32.46	78.94	SKINT9	-8.28	-3.39	PAQR7	-5.89		
IL6	9.64	GSTM1	-4.10	CCL20	6.22	TPM1	-2.76	IL1B	99.95	69.03	STFA3	17.13	71.60	MT4	-8.20	-3.69	FLG2	-10.54		
EDN1	9.15	KRTAP15	-4.07	FETUB	5.61	GABBR1	-2.59	CCL3	75.57	35.11	SPRR2E	43.69	71.19	BBOX1	-7.82	-6.86	BBOX1	-7.82		
DYNAP	8.58	SUSD2	-3.84	PINLYP	5.54	MERTK	-2.55	KLK6	75.19	50.58	IL1B	99.95	69.03	ANGPTL7	-7.74	-6.04	PVALB	-6.46		
TUBB2B	7.93	ABHD12B	-3.80	DEFB6	4.86	MYLK2	-2.53	LCE3A	72.49	51.58	2610528	64.33	63.23	SEMA3D	-7.33	-5.47	ANGPTL7	-7.74		
MMP1B	7.65	PRLR	-3.76	PAPL	4.79	RGS5	-2.49	PTGS2	66.98	19.91	DEFB4	113.92	56.46	CES1D	-7.14	-5.69	KRT15	-5.50		
BCL2L15	7.10	IL34	-3.73	RNASE2B	4.64	NBL1	-2.45	2610528	64.33	63.23	LCE3A	72.49	51.58	GM10024	-6.51	-3.72	ALOX12E	-9.00		
CXCR4	7.01	CLCA3A2	-3.66	IL19	4.11	ABLM3	-2.45	CXCL2	62.94	32.24	DEFB3	7.97	50.83	PVALB	-6.46	-6.35	CES1D	-7.14		
SLCO1A5	6.76	LGR6	-3.61	SPINK7	3.97	MEG3	-2.43	SAA3	58.46	29.39	KLK6	75.19	50.58	HLF	-6.29	-3.97	SEMA3D	-7.33		
TMPRSS11E	6.73	ACSBG1	-3.59	AWAT1	3.92	CD82	-2.43	KLK13	52.15	42.90	SPRR2D	36.44	46.44	FMO2	-6.12	-3.69	MYH4	-5.74		
CCRL2	5.75	KRT25	-3.49	ZC3H12A	3.89	LRRN1	-2.42	KRT6A	47.76	35.25	SPRR2B	4.10	45.69	TRDN	-6.06	-4.49	TRDN	-4.03		
TNFAIP6	5.70	SLURP1	-3.45	ELOVL3	3.82	TBX15	-2.42	SPRR1B	46.28	41.29	KLK13	52.15	42.90	LCE1M	-5.95	-4.29	TRDN	-5.72		
G530011	5.67	KRT2	-3.45	IL1F5	3.80	ENO3	-2.40	S100A8	45.57	99.59	SLC7A11	18.09	41.55	PAQR7	-5.89	-7.00	CASQ1	-4.75		
VPS37B	5.29	C130079	-3.41	CXCL13	3.62	SETBP1	-2.36	SPRR2E	43.69	71.19	SPRR1B	46.28	41.29	CILP	-5.77	-2.50	TRDN	-6.06		
TSLP	5.09	SKINT10	-3.40	FAM162A	3.62	TRPC1	-2.36	TREM1	43.34	24.11	KRT6A	47.76	35.25	MYH4	-5.74	-5.21	UCMA	-3.76		
B3GNT5	4.91	IL20RB	-3.39	AURKB	3.57	BICC1	-2.35	MMP13	40.06	2.05	CCL3	75.57	35.11	TRDN	-5.72	-5.12	TMOD4	-3.30		

FCH, Fold change.

pathways.⁴⁶ However, our results indicate that activation of p38 in the skin is sufficient to induce psoriatic inflammation. Inversely, mice lacking epidermal NF- κ B spontaneously have psoriatic skin lesions.²¹ Furthermore, there is no relationship between the activation efficiency of NF- κ B and the disease severity or phenotype of familial-type psoriasis with *CARD14* mutations,⁷ and *IL36RN* mutations in familial-type generalized pustular psoriasis are not fully related to the capacity to repress the NF- κ B activity.⁴⁷ Therefore development of psoriasis with *CARD14* or *IL36RN* mutations might not be due to activation of NF- κ B pathways but is likely due to excessive activation of p38 MAPK pathways in the skin.

Activation of p38 MAPK pathways in both epidermal keratinocytes and skin-resident DCs can be involved in the unique IL-17-mediated immune response and subsequent development of psoriatic dermatitis, although we have not clearly identified the cells targeted by anisomycin in this animal model. p38 signaling in Langerhans cells is expected to be involved in part in development of imiquimod-induced dermatitis,⁴⁸ and activation of p38 pathways in DCs drives T_H17 differentiation in patients with experimental autoimmune encephalomyelitis,⁴⁹

whereas activation of p38 in T cells, but not in macrophages or DCs, is critical for clearance of *Citrobacter rodentium* infection, which induces a strong local T_H17 response in the colon.⁵⁰ Moreover, UVB-induced inflammatory responses are suppressed by depletion of epidermal p38 but are intact in mice lacking the p38 gene in Langerhans cell or DETCs.⁵¹ These findings indicate the diverse contributions of p38 signaling in immune and nonimmune cells among organs and experimental animal models. However, our new animal model demonstrates that activation of p38 in the skin induces IL-17A-producing cells and is sufficient to induce dermatitis that is similar to psoriasis.

Despite the expected roles of p38 in patients with psoriasis, clinical studies have not demonstrated its essential role. Systemic p38 inhibitors did not show definitive clinical benefit in patients with psoriasis.³³ Consequently, our results indicate the possibility that cutaneous p38 could be a potential target for topical treatments in patients with psoriasis, although this has not yet been explored in clinical studies.

In summary, topical anisomycin treatment appears to capture the key features of psoriasis morphologically, immunologically, and clinically. In addition, inhibition of p38 MAPK suppresses

FIG 5. Transcriptomic profiling of anisomycin or imiquimod (IMQ)-induced dermatitis and human psoriasis. **A**, Heat map image of microarray analysis of RNA from mice ear skin after IMQ and anisomycin treatment compared with no inductor (n = 3). **B**, Venn diagram based on the differentially expressed gene in each model compared with the untreated controls illustrates the overlap and different profiles of these murine models. **C**, Expression levels of representative psoriasis-related genes increased in the anisomycin model, IMQ model, or both. **D**, Enrichment of cytokine-related inflammatory pathways (gene sets) in human psoriasis transcriptomes and the anisomycin and IMQ models of psoriasis. Normalized enrichment score (NES) and false discovery rate (FDR) are shown for gene sets regulated in keratinocytes (KC), reconstituted human epidermis (RHE), and fibroblasts by several psoriatic inflammatory cytokines.³⁵ Expression of these cytokine pathways in human psoriasis vulgaris is shown for lesional (LN) versus normal (NN) skin in the Gudjonsson transcriptome³⁶ and for lesional (LS) versus nonlesional (NL) skin in the Meta-Analysis-Derived 3 (MAD3) transcriptome.¹ Cytokine enrichment in the anisomycin and IMQ models is shown.

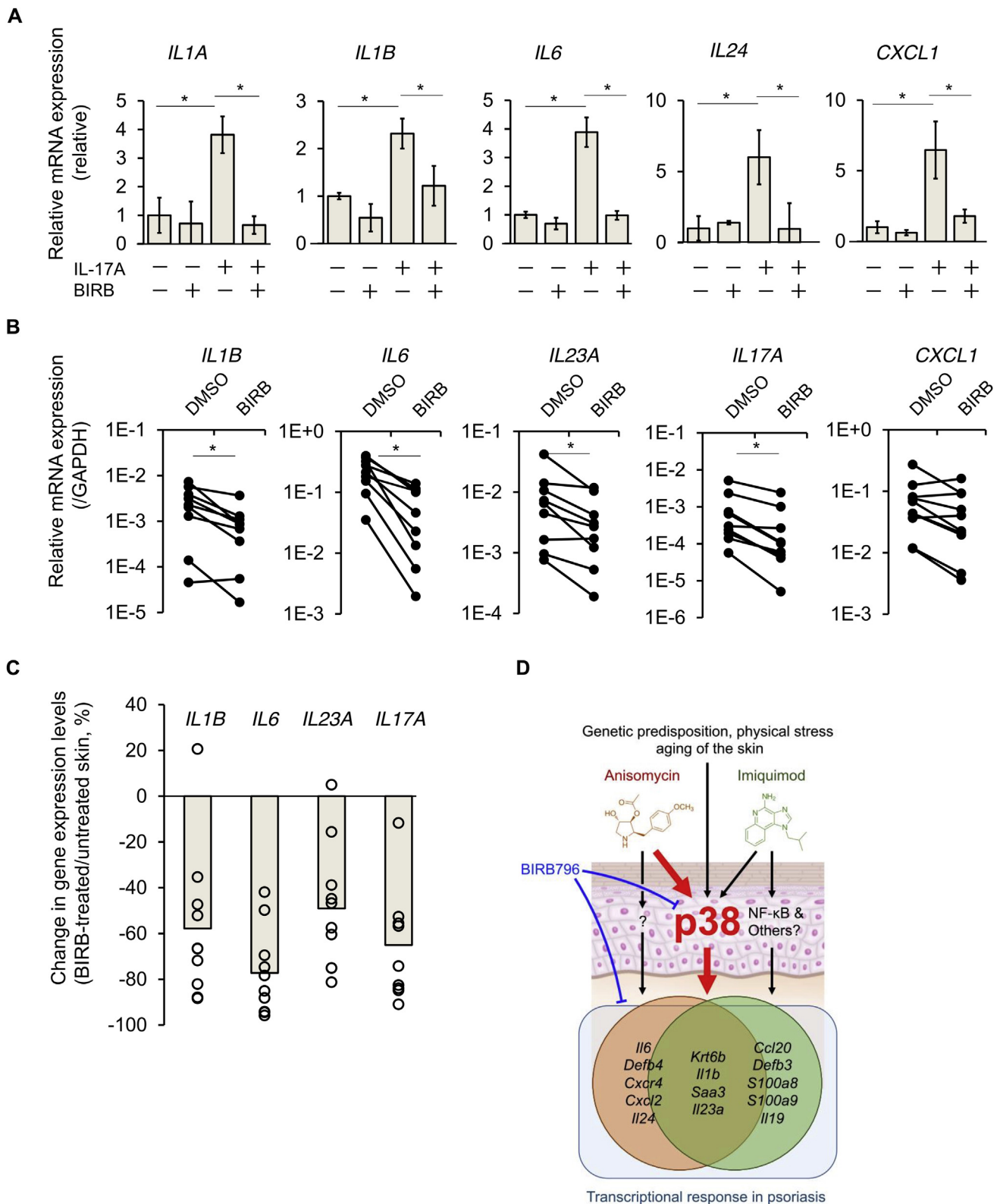


FIG 6. Effect of BIRB796 on NHEKs and human psoriatic skin. **A**, Quantitative RT-PCR analysis of mRNA levels in NHEKs treated with rIL-17A with or without BIRB796 for 2 hours ($n = 3$). Results were normalized to glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) expression and are shown as means $\pm$ SDs. $*P < .05$. **B**, Quantitative RT-PCR analysis of mRNA levels of inflammatory genes in lesional skin treated with or without BIRB796 for 12 hours *ex vivo*. Results were normalized to *GAPDH* expression, and a number that is the base of a logarithm is 10. Ten patients with psoriasis were recruited. $*P < .05$. **C**, Inflammatory gene expression percentage in lesional skin after BIRB796 treatment relative to vehicle treatment. **D**, Schematic diagrams of signaling pathways in anisomycin- or imiquimod-induced psoriasis animal models. Degrees of activation of the p38 and NF- κ B pathways are expected to give rise to differences in gene expression profiles between these 2 animal models.

expression of psoriasis-related cytokines in NHEKs and human psoriasis specimens. We propose that breakdown of skin homeostasis with overactivation of p38 MAPK simulates the cellular stresses that trigger psoriasis in predisposed subjects at the peak age, and the repositioning of topical p38 MAPK inhibitors might expand the currently unsatisfactory topical treatments for psoriasis.

Key messages

- Activation of p38 MAPK in the skin induces psoriatic inflammation.
- Cutaneous p38 MAPK is a therapeutic target of topical treatments for psoriasis.

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