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Paeoniflorin inhibits skin lesions in imiquimod-induced psoriasis-like mice by downregulating inflammation

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ABSTRACT

Psoriasis is a common chronic immune-mediated inflammatory disease. It is well known that macrophages, neutrophils and T-helper 1 (Th1)/T-helper 17 (Th17) cells play important roles in skin lesions by provoking inflammation. Paeoniflorin (PF) is the major effective component extracted from the root of *Paeonia lactiflora*, which has been widely used in China to treat inflammatory and autoimmune diseases, including psoriasis. Although PF shows a clinical therapeutic effect on psoriasis patients, how PF affects infiltrated immune cells in psoriasis skin lesions is still unknown. In this study, using a generated imiquimod (IMQ)-induced psoriasis-like mouse model, we found that PF ameliorates inflammation and skin lesions. Subsequent analyses showed that PF decreases the number of F4/80$^+$CD68$^+$ macrophages and their related cytokine production (TNF-α, IL-1β, IL-6, IL-12 and inducible nitric oxide synthase (iNOS)) in the skin of IMQ-challenged mice. Moreover, PF suppresses the number of CD11b$^+$Gr-1$^+$ neutrophils and the expression of macrophage inflammatory protein-2 (MIP-2; a counterpart of human IL-8, which is responsible for the recruitment of neutrophils in mice). Finally, PF also down-regulates Th1- and Th17-related cytokine expression. Therefore, our new findings reveal that PF alleviates psoriatic skin lesions by inhibiting inflammation, which provides new insights into the immunomodulatory effect of PF in psoriasis treatment.

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1 . Introduction

Psoriasis is a common chronic immune-mediated inflammatory disease, which has an overall prevalence of 2% to 3% in the general population [1]. Psoriasis has been associated with many comorbidities, including cardiovascular disease, obesity, diabetes, hypertension, dyslipidemia, metabolic syndrome, nonalcoholic fatty liver disease, cancer, anxiety and depression, inflammatory bowel disease and psoriatic arthritis [2]. The major histological features of psoriasis are epidermal hyperplasia with dysregulated keratinocyte differentiation, pronounced inflammatory cell infiltration and increased vascularization [3,4]. Although the pathogenesis is still unclear, it has been reported that macrophages, neutrophils and Th1/Th17 cells play important roles in psoriasis-related skin lesions by provoking inflammation [5–7]. It has been well established that prominent skin infiltration by neutrophils and microabscess formation is the hallmark feature of psoriasis [8].

Recently, neutrophils were reported to release not only pro-inflammatory factors but also IL-17 in psoriatic skin lesion [6]. Cutaneous macrophages, present in psoriatic lesions, can produce many mediators and cytokines (TNF-α, IL-1β and IL-6), which are thought to take part in the development of skin inflammation in psoriasis [5]. Many investigators have demonstrated that T-cell dysregulation is another pathogenesis of psoriasis because increased amounts of Th1/Th17 cells are contained in the psoriatic lesions [7]. Biological therapies (such as the TNF inhibitors etanercept, adalimumab and infliximab, as well as the IL-12/23 inhibitor ustekinumab) have proven to be highly effective in psoriasis treatment. However, high rates of adverse drug reactions and serious adverse events have been observed, including an increased propensity for viral infections [9,10].

Total glucosides of paeony (TGP) is an active compound extracted from the root of the Chinese peony, *Paeonia lactiflora*. Paeoniflorin (PF) is one of the principal bioactive components of TGP (more than 90%), which has been widely used in China for the treatment of inflammatory and autoimmune diseases, such as rheumatoid arthritis (RA), psoriasis, Sjögren's syndrome (SS) and systemic lupus erythematosus (SLE) [11–14]. It has been reported that PF decreases the number of Th1 and Th17 cells in the peripheral blood derived from RA patients, and it was found to inhibit the production of pro-inflammatory

Abbreviations: TGP, total glucosides of paeony; PF, paeoniflorin; RA, rheumatoid arthritis; SS, Sjögren's syndrome; SLE, systemic lupus erythematosus; CIA, collagen-induced arthritis; NOD, non-obese diabetic; IMQ, imiquimod; iNOS, inducible nitric oxide synthase; MIP-2, macrophage inflammatory protein-2; M1, classically activated macrophage; M2, alternatively activated macrophage.

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cytokines, such as TNF-α and IL-1β, in the synoviocytes of collagen-induced arthritis (CIA) rats [12,15]. Consistently, PF also decreased the Th1 numbers in the peripheral blood of psoriatic arthritis patients [11]. Moreover, PF increased saliva secretion in SS patients and delayed the onset of SS in non-obese diabetic (NOD) mice [13,16]. PF has also shown a clinical therapeutic effect on SLE patients by increasing the number of regulatory T cells (Treg) [14]. It has been proven that PF treatment shows little evident toxicity or side effects: PF combined with leflunomide and methotrexate reduced the hepatotoxicity for RA patients [17,18]; therefore, PF treatment is considered to be an effective therapy for inflammatory and autoimmune diseases. Although PF has shown a clinical therapeutic effect on psoriasis patients, how PF affects the infiltrated immune cells in psoriatic skin lesions is still unclear.

In this study, using a generated imiquimod (IMQ)-induced psoriasis-like mouse model, we analyzed the effect of PF on skin lesions *via* reducing inflammation. The results showed that PF ameliorated inflammation and the skin lesions. Subsequent analyses revealed that PF decreased the number of $F4/80^+CD68^+$ macrophages and $CD11b^+Gr\text{-}1^+$ neutrophils and their related cytokine production (TNF-α, IL-1β, IL-6, IL-12, IL-23, inducible nitric oxide synthase (iNOS) and macrophage inflammatory protein-2 (MIP-2)) in the skin of IMQ-challenged mice. Furthermore, PF also down-regulated Th1/Th17-associated cytokine production. Therefore, our new findings reveal that PF alleviates psoriatic skin lesions by inhibiting inflammation, providing new insights into the potential immunomodulatory effect of PF in psoriasis treatment.

2 . Material and methods

2.1 . Drugs

PF (molecular weight 480.05, Liwah Plant extract technology Co. Ltd, Ningbo, China, with a purity of 96%) was dissolved in double-distilled water and filtered. A total of 75, 150, or 300 mg/kg/day of PF was used to treat mice (i.p.) each day, according to previous studies [12,19].

2.2 . Animals

Female 8–11w Balb/c mice were purchased from the Shanghai Laboratory Animal Center, Chinese Academy of Science (Shanghai, China). The mice were maintained under pathogen-free conditions. All of the experiments were performed according to the Animal Care and Use Committee guidelines.

2.3 . IMQ-induced psoriasis-like mouse model

The mice received a daily topical dose of 60 mg/2.5 mg of 5% IMQ cream (Aldara; 3M Pharmaceuticals, Minnesota, USA) on the shaved back/right ear each day, according to a previous study [20]. The control mice were treated with vehicle cream (Fagron, Rotterdam, Netherlands) in the same way. After 6 days of IMQ administration, the psoriasis symptoms appeared on the backs and ears of the mice, and the ear thickness was measured with vernier calipers. For the PF treatment, the mice were treated with different doses of PF (75, 150, or 300 mg/kg/day) or with the vehicle control (H_2O) (i.p.) every day after the psoriasis symptoms appeared (day 6 after IMQ administration) for 10 consecutive days. Then, the mice were euthanized *via* cervical vertebral dislocation on day 16.

2.4 . H&E staining

The ears and back skin of the IMQ-challenged mice treated with 150 mg/kg/day of PF or the vehicle were removed from the euthanized mice and fixed in 4% paraformaldehyde solution and embedded in paraffin. The paraffin-embedded (5–10 μm) sections were stained with H&E and examined *via* light microscopy.

2.5 . RNA extraction and real-time PCR

A total of 10 mg of the back skins from the IMQ-challenged mice using different doses of PF (75, 150, or 300 mg/kg/day) or the vehicle was homogenized, and the total RNA was extracted using TRIzol reagent (Roche Applied Science, Penzberg, Germany). The messenger RNA (mRNA) was converted to cDNA using the RevertAid™ First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc., Massachusetts, USA). A two-step real-time PCR was performed using SYBR Green Master Mix (Applied Biosystems, California, USA) according to the manufacturer's instructions. The β-actin gene was used as the endogenous control. The gene expression was calculated as the difference in the cycle threshold (ΔCt) between the target gene and β-actin; ΔΔCt was the difference between the ΔCt values of the test sample and that of the control. The relative expression of the target genes was calculated as $2^{-\Delta\Delta Ct}$ as previously reported [21]. The primers were developed using Primer Express 2.0 software (Applied Biosystems) and are shown in Table 1.

2.6 . Flow cytometric analysis

The back skin from the IMQ-challenged mice using PF (150 mg/kg/day) or the vehicle were cut into pieces and digested using collagenase I (Sigma, Missouri, USA) for 2 h at 37 °C and then filtered the supernatants through a 0.22 μm low protein binding filter (Millipore, Massachusetts, USA). The collected cells were stained with APC-conjugated CD3, PE-Cy5-conjugated CD11b, PE-conjugated Ly-6G/Gr-1, PE-conjugated CD68 and PerCP-Cy5.5-conjugated F4/80 anti-mouse mAb (eBioscience Inc., California, USA). The flow cytometry was performed using a FACSCalibur cytometer (BD Biosciences) and was analyzed using FlowJo7.6 software (Treestar Inc., California, USA).

2.7 . Western blot analysis

A total of 30 mg of the back skin from the IMQ-treated mice using PF (150 mg/kg/day) or the vehicle were homogenized, and the cells were lysed. The expression of iNOS was detected using a rabbit anti-mouse iNOS antibody (Cell Signaling Technology Inc., Massachusetts, USA) as previously reported [21]. Briefly, the cell lysates were separated *via* SDS-PAGE electrophoresis and transferred to PVDF membranes

Table 1
Real-time PCR primers.

Name	Primer	Sequence(5′–3′)	Accession Numbers
β-actin	FW	TGTCCACCTTCCAGCAGATGT	NM_007393.3
	RV	AGCTCAGTAACAGTCCGCCTAG	
TNF-α	FW	CAGGCAGGTTCTGTCCCTTTC	NM_013693.3
	RV	CTGTGCTCATGGTGTCTTTTCTG	
INF-γ	FW	ATGAACGCTACACACTGCATC	NM_008337.3
	RV	CCATCCTTTTGCCAGTTCCTC	
iNOS	FW	GCCACCAACAATGGCAACA	NM_010927.3
	RV	CGTACCGGATGAGCTGTGAA	
IL-1β	FW	GAGCACCTTCTTTTCCTTCATCTT	NM_008361.3
	RV	TCACACACCAGCAGGTTATCATC	
IL-6	FW	TTCCATCCAGTTGCCTTCTTG	NM_031168.1
	RV	GGGAGTGGTATCCTCTGTGAAGTC	
IL-12	FW	AAGCTCTGCATCCTGCTTCAC	NM_001159424.1
	RV	GATAGCCCATCACCCTGTTGA	
IL-17	FW	GGTCAACCTCAAAGTCTTTAACTC	NM_010552.3
	RV	TTAAAAATGCAAGTAAGTTTGCTG	
IL-21	FW	CGCCTCCTGATTAGACTTCG	NM_021782.2
	RV	TGTTTCTTTCCTCCCCTCCT	
IL-22	FW	TCATCGGGGAGAAACTGTTC	NM_016971.2
	RV	GCTGATGTGACAGGAGCTGA	
IL-23	FW	AATAATGTGCCCCGTATCCA	NM_031252.2
	RV	CATGGGGCTATCAGGGAGTA	
MIP-2	FW	CCCTCAACGGAAGAACCAAA	NM_009140.2
	RV	AGGCACATCAGGTACGATCCA	

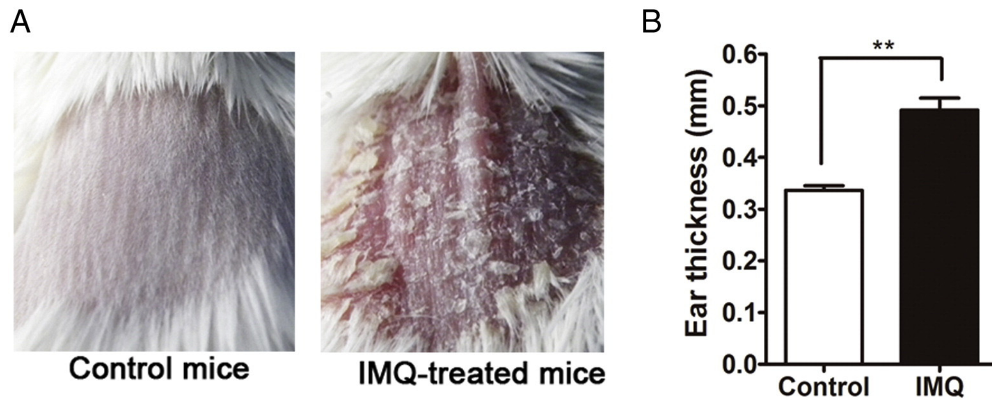

Fig. 1. Generation of the IMQ-induced psoriasis-like mice. (A) The mice received a daily topical dose of 60 mg of 5% IMQ cream or Vaseline cream on their skin (as a control) for 6 consecutive days. (B) The ear thickness of the mice challenged with IMQ or the control was measured on day 6 using vernier calipers (n = 8). The data represent one of the three independent experiments. The data represent the mean ± SEM of at least three independent experiments. ** = $p < 0.01$ *vs.* the control group.

(Millipore) at 90 V for 2 h. The membrane was blocked with 5% non-fat milk, washed with PBST (PBS containing 0.1% Tween 20), and incubated with mAbs at 4 °C overnight. Then, the membrane was incubated with an HRP-conjugated goat anti-rabbit IgG (Cell Signaling Technology Inc.) at room temperature for 1 h followed by washing with PBST. The target proteins were examined using the ECL system (Millipore) and visualized using autoradiography film. A Gel-Pro Analyzer 4 (Exon--Intron Inc., Pennsylvania, USA) was used to analyze the density of the WB results. The alteration of the target protein was displayed as relative fold-change, which was derived by comparing with β-actin.

2.8 . NO synthesis measurement

A total of 30 mg of the back skin from the IMQ-treated mice using PF (150 mg/kg/day) or the vehicle were homogenized, and the cells were lysed. The supernatant was collected and the NO levels were examined using a commercial Nitric Oxide Assay Kit (Beyotime Biotech Inc., Jiangsu, China). Briefly, 50 μl of Griess reagent I and 50 μl of Griess reagent II were added to 50 μl of the supernatant in a 96-well plate. The nitrite concentration was evaluated according to the absorbance at 540 nm using a microplate reader (Bio-Rad, California, USA).

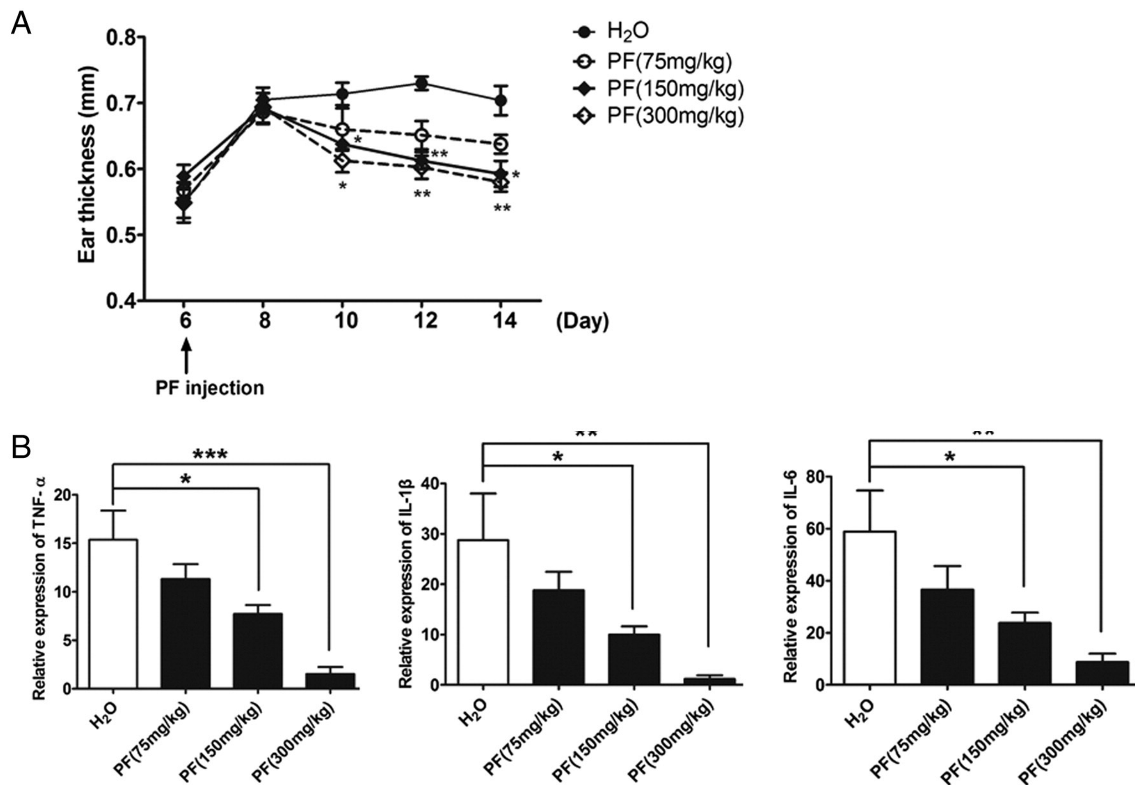

Fig. 2. The efficacy of the different concentrations of PF on the IMQ-challenged mice. (A) The ear thickness of the IMQ-challenged mice treated with the different concentrations of PF (75 mg/kg/day (○), 150 mg/kg/day (◆), or 300 mg/kg/day ( )) or the vehicle (●), which was measured every other day using vernier calipers (n = 8). (B) The mRNA expression of TNF-α, IL-1β and IL-6 in the skin from the PF-treated (black bars) or vehicle-treated (open bars) mice was evaluated *via* real-time PCR (n = 8). The data represent the mean ± SEM of at least three independent experiments. * = $p < 0.05$, ** = $p < 0.01$ *vs.* the H_2O group.

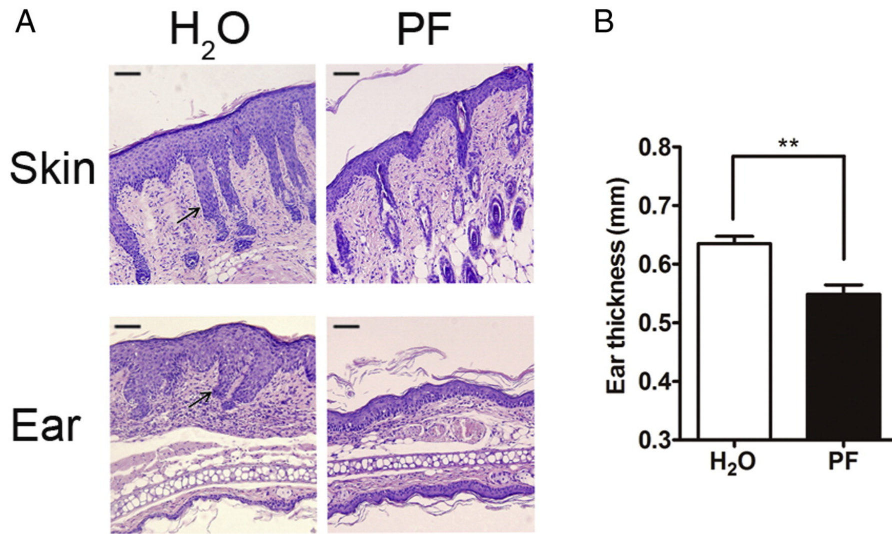

Fig. 3. PF alleviated the psoriatic symptoms and skin inflammation in the IMQ-treated mice. (A) The histopathology of the skin and ear tissue sections of the IMQ-treated mice following PF (150 mg/kg/day) or vehicle administration *via* H&E staining. Original magnification, 200×. Bar, 20 μm. The arrows indicate the downward extension of the epidermal rete ridges. (B) The ear thickness was measured at the end of the PF (150 mg/kg/day) or vehicle (H_2O) treatment (n = 6). The data represent the mean ± SEM of at least three independent experiments. ** = p < 0.01 *vs.* the H_2O group.

2.9 . Statistical analysis

All of the experiments were performed in triplicate. The difference among the groups was determined *via* ANOVA analysis, and the comparison between two groups was analyzed by *t*-test using GraphPad Prism 5.0 (GraphPad Software Inc., California, USA). A value of $P < 0.05$ was considered to be statistically significant.

3 . Results

3.1 . PF alleviates inflammation and skin lesions in IMQ-induced psoriasis-like mice

PF has been reported to show a clinical therapeutic effect on psoriasis in China. To investigate the potential mechanism of PF on psoriasis

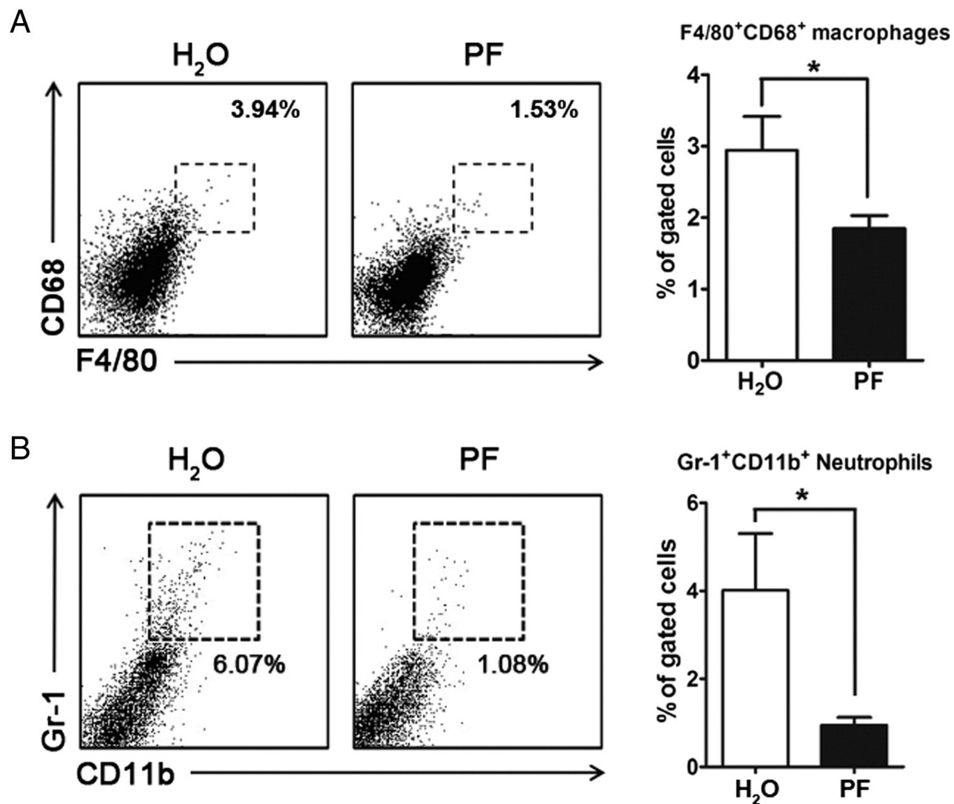

Fig. 4. PF affects the number of macrophages and neutrophils *in vivo*. The populations of $F4/80^+CD68^+$ macrophages (A) and $CD11b^+Gr\text{-}1^+$ neutrophils (B) in the skin derived from the IMQ-challenged mice following PF (black bars) or vehicle (open bars) administration, detected *via* flow cytometry (n = 8). The data represent the mean ± SEM of at least three independent experiments. * = p < 0.05 *vs.* the H_2O group.

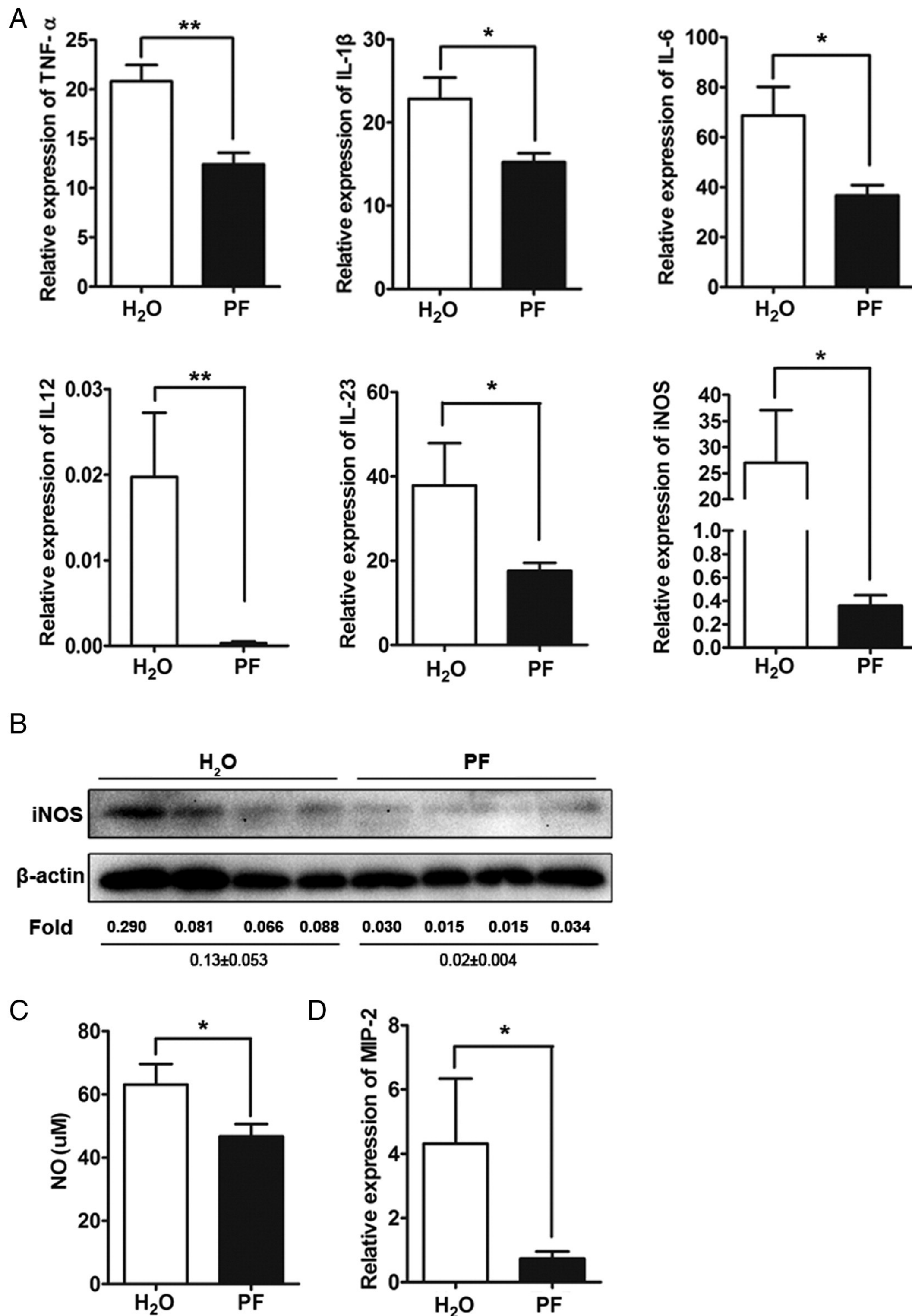

Fig. 5. PF inhibits macrophage- and neutrophil-related cytokine production. (A) The mRNA expression of TNF-α, IL-1β, IL-6, IL-12, IL-23 and iNOS in the skin from the PF-treated (black bars) or vehicle-treated (open bars) mice, evaluated *via* real-time PCR (n = 8). (B) The iNOS protein expression in the skin lysates of the PF- or vehicle-treated mice examined *via* western blotting. The alteration in iNOS was displayed as the relative fold change that was derived from the comparison with β-actin. (C) The NO production of the skin from the PF-treated (black bars) or vehicle-treated (open bars) mice evaluated using a nitrite detection kit (n = 8). (D) The MIP-2 expression in the skin from the PF-treated (black bars) or vehicle-treated (open bars) mice evaluated *via* real-time PCR (n = 8). The data are representative of at least three independent experiments. * = $p < 0.05$, ** = $p < 0.01$ *vs.* the H_2O group.

treatment *in vivo*, we first generated an IMQ-induced psoriasis-like mouse model. After treatment with IMQ for 6 consecutive days, the mice showed an apparent psoriasis phenotype, including erythema, scaling and thickening of the skin (Fig. 1A) and remarkably thickened ears (Fig. 1B, measured using vernier calipers). These indicate that we successfully established an IMQ-induced psoriasis-like mouse model in concert with previous report [20].

Subsequently, we tested the efficacy of PF treatment at different doses (75, 150, or 300 mg/kg/day, i.p.) in the IMQ-challenged mice. The results showed that the ear thickness was reduced in the mice

treated with 150 and 300 mg/kg of PF (Fig. 2A). Consistently, the expression of the inflammatory cytokines (TNF-α, IL-1β and IL-6) in the skin of the IMQ-challenged mice was also alleviated by the 150- and 300-mg/kg PF treatment (Fig. 2B), as detected *via* real-time PCR. Considering that 150 mg/kg/day of PF is the equivalent dose used in clinical treatments [19], we treated the mice with this dose of PF in the following studies. The histopathology assay revealed that PF ameliorated the skin inflammation, reduced the epidermis thickening, diminished the downward extension of the epidermal rete ridges and inhibited the brisk cellular infiltration of the mononuclear cells in the dermis (Fig. 3A). Moreover, PF dramatically inhibited the ear thickness after 16 days of IMQ application (Fig. 3B). These data show that PF relieves the apparent inflammation and skin lesions in IMQ-treated mice.

3.2 . PF decreases the macrophage and neutrophil populations in vivo

Given that macrophages and neutrophils are reported to release amounts of pro-inflammatory factors, which are thought to take part in the development of skin inflammation in psoriasis [5,6], we first examined the effect of PF on the macrophage and neutrophil populations in the IMQ-induced mice. We cut the skin samples from the IMQ-treated mice into pieces, digested them using collagenase and examined the profiles of the dermal macrophages and neutrophils *via* flow cytometry. The results showed that, compared with the PF-untreated group, PF decreased the number of F4/80$^+$CD68$^+$ macrophages and CD11b$^+$Gr-1$^+$ neutrophils significantly in the skin from the IMQ-treated mice (Fig. 4A and B). These results indicate that PF diminishes macrophage and neutrophil populations *in vivo*.

3.3 . PF suppresses macrophage- and neutrophil-related cytokine production in IMQ-induced mice

It is well established that TNF-α, IL-1β, IL-6, IL-12, IL-23 and iNOS are abundantly present in psoriasis patients [22,23], and macrophages are known to produce these cytokines [24]. As we have previously shown that PF decreases the number of F4/80$^+$CD68$^+$ macrophages, to further explore whether PF inhibits the cytokine production by these macrophages, we analyzed the macrophage-related cytokine profiles in the skin from IMQ-induced mice using a real-time PCR assay. The results showed that the levels of TNF-α, IL-1β, IL-6, IL-12, IL-23 and iNOS were significantly reduced in the PF-treated mice (Fig. 5A). Moreover, we examined the iNOS protein levels in the mice *via* a western blot assay and found that PF decreased the iNOS production in the IMQ-challenged mice (Fig. 5B), which is consistent with the altered iNOS mRNA expression. To further evaluate the enzymatic activity of iNOS, we also detected the NO production in the skin of the IMQ-challenged mice using a Nitric Oxide Assay Kit, and the results showed that the NO levels were diminished by the PF treatment (Fig. 5C).

As microabscess formation (due to the presence of infiltrated neutrophils in psoriatic skin) is the hallmark feature of psoriasis, and MIP-2 (a counterpart of human IL-8) plays a pivotal role in the recruitment and activation of neutrophils in mice [8,25], we subsequently examined the MIP-2 expression affected by PF using a real-time PCR assay. The results showed that the PF treatment led to a reduction in MIP-2 expression (Fig. 5D), which may decrease the infiltration by the neutrophils. Based on these results, we suggest that PF alleviates skin lesions by inhibiting not only the number of macrophages and neutrophils but also *via* their cytokine production during psoriasis treatment.

3.4 . PF inhibits Th1/Th17-associated cytokine production in vivo

It is suggested that downregulating the function of Th1/Th17 cells is an effective therapy for psoriasis [26,27]; therefore, we further evaluated whether PF affects the Th1/Th17-associated cytokine expression *in vivo*. First, we found that the number of dermal CD3$^+$ T cells was decreased in the IMQ-challenged mice that were treated with PF, as shown *via* flow cytometry (Fig. 6A and B). Because of the limited cell number of cells derived from the lesion skins and considering that Th1 and Th17 cells play crucial roles in the pathogenesis of psoriasis, we examined the mRNA expression of the Th1/Th17-associated cytokines in the psoriatic skins *via* real-time PCR. The results showed that the INF-γ mRNA expressed by the dermal Th1 cells was significantly decreased (Fig. 7A). The IL-17, IL-21 and IL-22 mRNAs expressed by the dermal Th17 cells were reduced in the PF-treated mice compared with the control mice (Fig. 7B–D). Together, these data suggest that PF suppresses the erosion of the skin in IMQ-treated mice by attenuating INF-γ, IL-17, IL-21 and IL-22 production, indicating that PF might affect the inflammation induced by dermal Th1/Th17 cells during psoriasis treatment.

4 . Discussion

Psoriasis is considered to be an inflammatory-associated disease. Numerous studies have demonstrated that cutaneous macrophages, neutrophils and Th1/Th17 cells play vital roles in psoriatic skin lesion by provoking inflammation [5–7]. PF has been widely used in China to treat inflammatory and autoimmune diseases, including psoriasis [11,12,14,16]. Mechanistically, PF reduces the activation of Th1 and Th17 cells and decreases TNF-α, IFN-γ and IL-2 expression in RA patients and in CIA mice [12,15]. Consistently, PF inhibits dendritic cell (DC) maturation and reduces IL-6 and IL-12 production in CIA mice [12]. Moreover, PF suppresses the activation of macrophages in CIA and

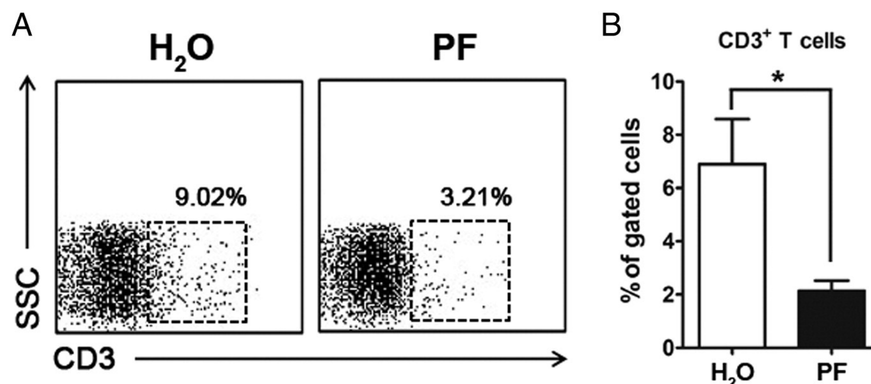

Fig. 6. PF suppresses the CD3$^+$ T-cell profile *in vivo*. (A) The proportion of CD3$^+$ T cells in the skin derived from the IMQ-challenged mice with or without PF treatment detected *via* flow cytometry. (B) The percentage of CD3$^+$ T cells in the skin derived from the IMQ-challenged mice following PF (black bars) or vehicle (open bars) administration, detected *via* flow cytometry (n = 8). The data represent the mean ± SEM of at least three independent experiments. * = $p < 0.05$ *vs.* the H_2O group.

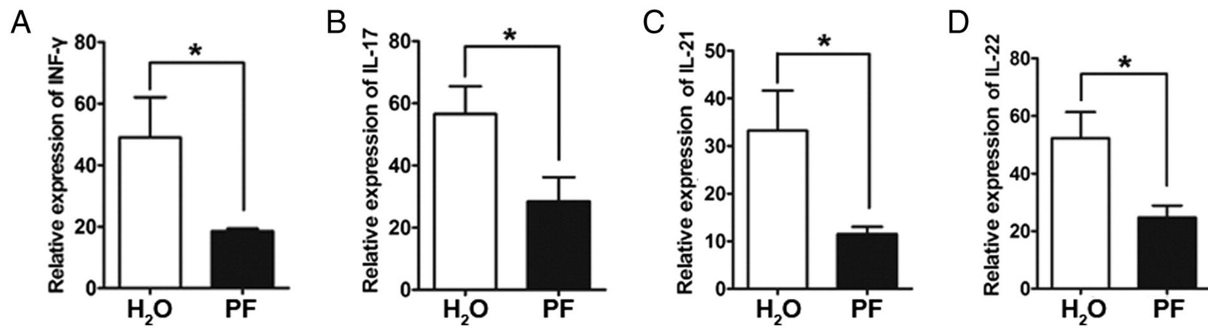

Fig. 7. PF diminishes the Th1 and Th17 cell-related cytokine production. The mRNA expression of INF-γ (A), IL-17 (B), IL-21 (C) and IL-22 (D) in the skin from the PF-treated (black bars) or vehicle-treated (open bars) mice evaluated *via* real-time PCR (n = 8). The data are representative of at least three independent experiments. * = $p < 0.05$ *vs.* the H_2O group.

diabetic rats [28,29]. Recently, a gene array assay showed that PF also decreases inflammatory cytokine expression [30].

Using an IMQ-induced psoriasis-like mouse model, we explored the potential mechanism of PF on psoriasis treatment in this study. The results showed that PF alleviated psoriatic skin lesions by reducing the number of inflammatory cells and their related cytokine production.

Macrophages are involved in the pathogenesis of inflammatory and autoimmune diseases, such as RA and psoriasis [5,31]. Numerous studies have found that macrophages accumulate in psoriatic skin lesions and release a range of cytokines, such as TNF-α, IL-1β, IL-6 and IL-12, leading to the excessive proliferation of keratinocytes and the skin inflammation observed in psoriasis [32–34]. Therefore, attenuating macrophage function has been proposed as an effective strategy for psoriasis treatment [35,36]. TNF-α is one of the major inflammatory cytokines produced by macrophages, and it is involved in the pathogenesis of psoriasis and is considered to be an important biological treatment target for psoriasis [35,36]. TNF-α induces the keratinocytes to express intercellular adhesionmolecule-1 (ICAM-1) and pro-inflammatory cytokines (such as IL-1β, IL-6 and IL-8) [37,38]. It is well established that IL-12 supports the activation and differentiation of Th1 cells, and IL-1β, IL-6 and IL-23 contribute to Th17 differentiation [39,40]. In the current study, we found that PF decreased the number of F4/80$^+$ macrophages and inhibited the expression of TNF-α, IL-1β, IL-6, IL-12, IL-23 and iNOS in the skin lesions, suggesting that PF not only decreases the number of macrophages but also inhibits macrophage function, which could further suppress the Th1/Th17-associated inflammation and skin lesions.

Resent researches have made it clear that macrophages are classified into classically activated (M1) and alternatively activated (M2) phenotypes according to their differentiation and function [41,42]. M1 cells produce high levels of IL-12 and iNOS but low levels of IL-10, whereas M2 cells release large amounts of IL-10 and arginase-1 but low levels of IL-12. Therefore, M1 cells are known to be inflammatory inducers and promote tissue destruction, while M2 cells act as anti-inflammatory factors and contribute to tissue repair in diseases [42, 43]. As PF decreases the number of dermal macrophages and the level of IL-12 and iNOS expression and activity, we suggest that PF inhibits M1 activation in the skin lesions of IMQ-induced psoriasis-like mice.

It is well known that prominent skin infiltration by neutrophils and microabscess formation is the hallmark feature of psoriasis [8]. In addition, neutrophils are reported to release amounts of IL-17 [6]. Moreover, IL-8 (its counterpart in mice is MIP-2) is a direct chemoattractant for neutrophils. In this study, we found that PF reduced the population of neutrophils and the MIP-2 expression in the IMQ-administered mice, indicating that PF can suppress the number of infiltrated neutrophils and block the recruitment of neutrophils to the skin lesions.

Studies have shown that IFN-γ, which is produced primarily by the Th1 cells, exerts inflammatory effects in psoriasis by promoting antigen presentation and adhesion molecule expression by the keratinocytes [37,44]. Consistently, the Th17-associated cytokines IL-17, IL-21 and IL-22 have been found to be overexpressed in psoriatic skin and can cause epidermal hyperplasia and inflammation [45,46]. In the pathogenesis of psoriasis, activated dermal Th1 cells produce IFN-γ, which induces CXCL9, CXCL10 and CXCL11 synthesis, which can further recruit additional Th1 cells. Likewise, resident Th17 cells release IL-17 and IL-22 and activate the synthesis of chemokines (CCL20, CXCL1, CXCL2, and CXCL8/IL-8), leading to the recruitment of additional Th17 cells and neutrophils into the skin lesion [47]. Given that these processes amplify the inflammation and tissue erosion found in psoriasis, PF could slow down the symptoms of psoriasis by reducing the expression of IFN-γ, IL-17, IL-21 and IL-22.

Previously, the increased IL-17-producing cells found in psoriatic lesions were considered to only be due to Th17 cells, but it was recently reported that neutrophils are another main source of IL-17 [6]. Therefore, in the current study, PF was found to decrease the expression of IL-17 produced not only by the Th17 cells but also by the neutrophils, which is consistent with the reduced number of neutrophils in the IMQ-induced psoriasis-like mice treated with PF.

Recently, biological therapies using TNF inhibitors, IL-12/23 inhibitors, as well as IL-17 inhibitors have been proven to be highly effective for treating psoriasis. It is worth mentioning that PF can suppress the targeted cytokine expressed in psoriatic skin lesions, implicating that PF may be beneficial for effective psoriasis treatment.

In summary, the current study showed that PF ameliorates psoriatic skin lesions and inflammation by decreasing the population of dermal macrophages, neutrophils and Th1/Th17-associated cytokine production in the skin of IMQ-challenged mice. As epidermal hyperplasia and inflammation are the hallmarks of psoriasis, our new finding reveals that PF treatment not only alleviates these skin lesions by inhibiting inflammation but also relieves epidermal hyperplasia by reducing the expression of TNF-α, IL-17, IL-21 and IL-22, providing new insights into the potential immunomodulatory effects of PF in psoriasis treatment.

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