



Paeoniflorin attenuates the allergic contact dermatitis response via inhibiting the IFN- γ production and the NF- κ B/I κ B α signaling pathway in T lymphocytes

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ABSTRACT

Paeoniflorin (PF) has been demonstrated to have an anti-allergic and anti-inflammatory effect in the treatment of allergic contact dermatitis (ACD). However, its clinical application is hampered by the lacking of comprehensive mechanical explanation. This research aimed to study the effect of PF on the proliferation, apoptosis and cytokines secretion as well as the expression of nuclear factor-kappa B (NF- κ B) and mitogen-activated protein kinase (MAPK) signaling pathways of T lymphocytes activation *in vitro* and *in vivo*. We found that PF depressed human T lymphocytes activation via inhibition of interferon-gamma (IFN- γ) production and NF- κ B/I κ B α and p38 MAPK signaling pathway *in vitro*, also PF could attenuate such ACD responses by inhibiting the production of IFN- γ and NF- κ B/I κ B α pathway in T lymphocytes of ACD mouse model, suggesting that PF might be useful for the treatment of T cell-mediated allergic inflammatory disorders such as ACD. This would make PF a promising T cell-targeted drug candidate for further study because of its immunosuppressive and anti-inflammatory effects.

1. Introduction

Allergic contact dermatitis (ACD) is a very common inflammatory skin disease characterized by erythema, blisters and itching. It is caused by T cell-driven delayed-type contact hypersensitivity, affecting up to 20% of the general population [1]. Despite its increasing prevalence worldwide, the existing therapies, such as anti-allergic agents and steroid therapy, can not provide long-term remission and even many have severe side effects. Specific therapies for ACD are still limited due to complex pathogenic factors [2]. Natural herbs or oriental medicines, especially for their medicine monomers, have drawn much interest in the treatment of ACD for distinctive anti-allergic and immunomodulatory effects, including paeoniflorin, achyranthis radix extract and Kochia scoparia [3–5]. However, the underlying mechanisms of these commonly used therapies have not been clearly elucidated.

During inflammation reaction, activated T cells express higher levels of proinflammatory cytokines, such as interleukins (IL), tumor necrosis factor α (TNF- α) and interferon-gamma (IFN- γ) [6,7]. Excess cytokines

production leads to disturbance in epidermis, initiating inflammation symptoms. These cytokines are also known to induce inflammatory reaction cascades by different inflammatory signaling pathways that ultimately activate transcription factors and initiate cell proliferation and cytokine production [8,9]. Among these interrelated signaling pathways, signaling through nuclear factor κ B (NF- κ B) serves as a master regulator. NF- κ B is known to be a ubiquitous transcription factor complex. The activation of NF- κ B induces an ever-increasing list of target genes, which involve manifold biological activities including cell growth, survival and the immune response. Given their central role of T lymphocytes in modulating immune responses, targeting T lymphocytes activation and function is considered a promising mean of accomplishing therapeutic immunosuppression.

Paeoniflorin (C₂₃H₂₈O₁₁; PF; Fig. 1a) is the principal active component of total glucosides of paeony, which extracted from peony root and has been approved by the State Food and Drug Administration of China for rheumatoid arthritis (RA) treatment since 1998 [10]. Our previous study proved that a traditional herbal prescription Fuling Decoctions

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containing *Paeoniae radix*, which has been used for more than 10 years in our hospital, exhibited an efficient treatment for eczema patients via anti-type IV allergic activity and was less toxic compared with antihistamine [11]. Furthermore, through an analytical method incorporating ultra-fast liquid chromatography (UFLC) with mass spectrometry (MS) and ultraviolet (UV) detection which was developed for rapid profiling of the chemical constituents, we identified PF as one of the main components with anti-inflammatory bioactivity of Fuling Decoctions [12].

PF is reported to have anti-inflammation and immune-modulation effects on various types of cells by inhibiting cell proliferation, activation and cytokine production [3,13]. Based on T cell-mediated cytokine imbalances and their related activation of transcriptional factors in the pathogenesis of ACD, it is logical to postulate PF plays a role in the

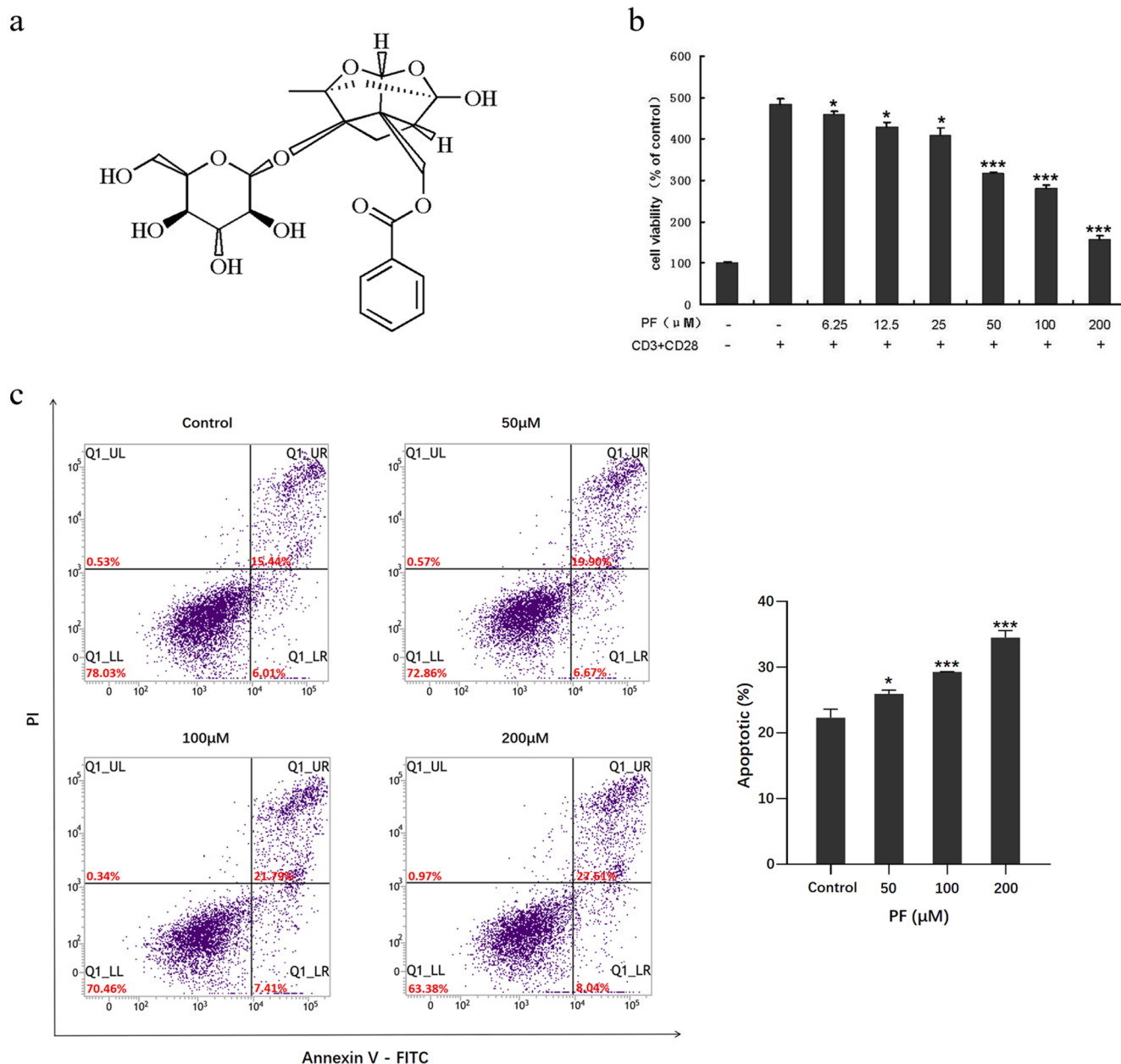
regulation of cytokine imbalance and the inhibition of transcription factor activation in T lymphocytes.

The current study aimed to investigate the effects of PF on activated T lymphocytes and to discover possible mechanisms involved in NF- κ B or mitogen-activated protein kinase (MAPK) signaling pathway. These may partially explain the action mechanism of PF in the treatment of ACD and provide new insights for the targeted therapy of ACD.

2. Materials and methods

2.1. Chemicals and antibodies

Paeoniflorin (MW: 480, purity > 98% by HPLC) was obtained from



Chengdu Pufei De Biotech Co Ltd (Chengdu, China). 1-chloro-2,4-dinitrobenzene (DNCB) and dimethyl sulfoxide (DMSO) were obtained from Sigma Chemical Co (USA). The antibodies for Western blot were purchased from Cell Signaling Technology (USA). The antibodies for Flow cytometry assay were purchased from BD Biosciences (USA).

The enzyme-linked immunosorbent assay (ELISA) kits for mice IL-4 and IFN- γ were products of R&D Systems (Bio-Techne, China), while human IL-4 and IFN- γ were products of Biosource International (Camarillo, CA, USA).

2.2. Cell cultures

Human peripheral blood mononuclear cells (PBMC) were isolated from the blood of healthy human blood donors according to a standard protocol using Ficoll-Hypaque (Pharmacia Biotech Inc., Piscataway, NJ, USA) density gradient centrifugation. Unfractionated mononuclear cells were separated into an E-rosette-positive (E1) population and were used as resting T lymphocytes. Monocytes were depleted by adherence to plastic plates for 1 h at 37 °C. In this study, each experiment was conducted with T lymphocytes cultured from three different donors.

The purity of T lymphocytes isolated in the above preparations was above 95%, which was determined by flow cytometry for CD3 antigen (data not shown). T cells were maintained in complete RPMI 1640 medium, supplemented with 10% FBS, 100 IU/mL penicillin and 100 μ g/mL streptomycin (Invitrogen, Carlsbad, CA, USA), then were cultured in a humidified incubator in 5% CO₂ at 37 °C.

2.3. Measurement of cell viability

Human T lymphocytes viability was evaluated by using the sulforhodamine B (SRB) assay as described previously [14]. Highly purified T lymphocytes were seeded in 96-well flat-bottomed plates at 1×10^6 cell/mL in 100 μ L of 10% FBS RPMI 1640 medium, 100 μ L anti-CD3 mAb and anti-CD28 mAb were added triplicately, and the final concentration of anti-CD3 mAb and anti-CD28 mAb were 2 μ g/mL and 5 μ g/mL respectively. Different concentrations (6.25, 12.5, 25, 50, 100, 200 μ M) of PF were incubated with activated T lymphocytes for 72 h. Cells were cultured in a humidified incubator in 5% CO₂ at 37 °C. At the end of the incubation, the cells were fixed with 10% trichloroacetic acid for 1 h at 4 °C, and the plate was washed 5 times with water and dried. 40 μ L of 0.4% SRB(W/V) solution were then added to the wells and incubated for 30 min, rinsed four times with 1% acetic acid, dried and added 10 mM Tris (pH 10.5) with 100 μ L per well. Read the absorbance value at 570 nm on spectrophotometric plate reader. Cell vitality was calculated using the formula: $100\% \times (\text{mean OD value of treatment group} / \text{mean OD value of control group})$.

2.4. Flow cytometry assay

Cell apoptosis was determined using a FITC Annexin V apoptosis kit (Elabscience, Wuhan, China). Briefly, different concentrations (50, 100 or 200 μ M) of PF were cultured with activated human T lymphocytes. Then the cells were harvested in 96-well flat-bottomed plates at 4×10^6 cell/mL in 50 μ L in a humidified incubator in 5% CO₂ at 37 °C for 72 h, and labelled with FITC-conjugated annexin-V and propidium iodide (PI) before detection with flow cytometry (BD FACSVerser, CA, USA).

For CD25, CD69 and NF- κ B detection, different concentrations (25, 50, 100 or 200 μ M) of PF were incubated with activated T lymphocytes for 48 h, then the cells were harvested and analyzed on flow cytometer.

2.5. Mice and treatment

The present study was approved by the Ethical Committee on Animal Research of Dalian Medical University (approved number: AEE19070). Balb/c female mice (18 ± 2 g, 6–8 weeks) were bred in the SPF-level Laboratory Animal Center of Dalian Medical University (Dalian,

China). The experimental animals were maintained in an animal facility under standard laboratory conditions.

DNCB-induced ACD was achieved as the previously published method [15]. Briefly, the mice were sensitized by painting about 4 cm² of shaved abdomen skin with 100 μ L of 1% DNCB solution (dissolved in a 4:1 mixture of acetone: olive oil). Five days later, the elicitation phase was induced by painting both sides of right ear with 20 μ L of 0.5% DNCB solution and rechallenged after 1 day, which resulted in extensive disease. 24 h after the second challenge, the spleen was obtained for T lymphocytes as described below.

The mice were randomly divided into 5 groups. The mice treated with DNCB were administered PF (75 and 150 mg/kg/day) and Hydrocortisone (Hyd) (24 mg/kg/day) orally from day 1 to day 7 after immunization. Normal group and ACD model group were given an equal volume of physiological saline.

Mice T lymphocytes were separated from spleens using Pan T Cell Isolation Kit (Miltenyi Biotec, USA) following the manufacturer's instructions. Mice T lymphocytes viability of different group was evaluated by using the MTT assay. Highly purified T lymphocytes were seeded in 96-well flat-bottomed plates at 1×10^6 cell/mL in 100 μ L of 10% FBS RPMI 1640 medium, 100 μ L ConA was added and the final concentration was 10 μ g/mL. Cells cultured in a humidified incubator in 5% CO₂ at 37 °C for 48 h. Four hours before the end of the incubation period, 20 μ L of MTT (5 mg/mL) was added to each well. DMSO was used to dissolve the formed formazan particles and read the absorbance value at 490 nm on spectrophotometric plate reader. Cell vitality was calculated using the formula: $\text{mean OD value of ConA treatment group} / \text{mean OD value of control group}$.

2.6. Cytokine production assays (IL-4, IFN- γ)

T lymphocytes were prepared in the same manner as described above. We chose 72 h to measure the cytokines produced by human T lymphocytes, which cytokines production were assayed in triplicate in 96-well, flat-bottomed plates as described above. The mice T lymphocytes were then collected and suspended in 10% FBS RPMI 1640 medium at a concentration of 1×10^7 cells/mL. The cell suspensions (500 μ L) and ConA (500 μ L with a final concentration of 10 μ g/mL) were simultaneously seeded into a 24-well culture plate. The cultures were incubated at 37 °C in an atmosphere of 5% CO₂ for 48 h. The supernatant was collected and reserved at -20 °C.

The concentrations of IL-4 and IFN- γ in T lymphocyte supernatant were measured using ELISA kits according to the manufacturer's instructions.

2.7. Western blotting

Purified human T lymphocytes (2×10^5 cells/mL) were transferred into 6-well plates and incubated with or without different concentrations of PF (1, 5 or 10 μ M) for 1 h, then were activated by CD3 (0.5 μ g/mL) combined with CD28 (1 μ g/mL) monoclonal antibody. T lymphocytes were collected at the optimal activation time points of the related proteins (data not shown, 120 min for NF- κ B; 60 min for p-IkB α ; 10 min for p-38, p-erk and p-jnk). The purified ACD mice T lymphocytes were collected as before.

Total protein extracts from T lymphocytes were obtained by using the Whole Cell Lysis Assay (KeyGEN, Nanjing, China), following manufacturer instructions. The protein concentrations of the cell lysates in supernatant were determined by using a BCA protein kit (KeyGEN, Nanjing, China), then the protein extracts were used for Western blotting analysis. Proteins were separated on 10% SDS-PAGE (EpiZyme, Shanghai, China) and then transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad, USA). The membranes were blocked with 5% skimmed milk or BSA for 2 h, followed by overnight incubation at 4 °C with specific antibodies against NF- κ B p65, P-IkB α (Ser32), IkB α , p-ERK (Thr202/Tyr204), ERK, p-JNK (Thr183/Tyr185), JNK, p-p38

(Thr180/Tyr182) and p38 (Cell Signaling Technology, USA). HRP-conjugated secondary antibodies (Cell Signaling Technology, USA) were added at a 1:2000 dilution for 1 h at room temperature. The immunoreactive proteins were detected by using ECL FemtoLight kit (EpiZyme, Shanghai, China) with Tanon 5200. The β -actin antibody (ABclonal, China) was performed under the same conditions as control.

2.8. Statistical analysis

Results are expressed as means $\pm$ SD, one-way ANOVA was used to compare the mean. SPSS 24.0 for Windows software package was used for data analysis. Statistical probability of $P < 0.05$ was considered statistically significant.

3. Results

3.1. PF prevented the proliferation and promoted the apoptosis of activated T lymphocytes

To investigate the effect of PF on T lymphocytes proliferation, we performed the sulforhodamine B (SRB) assay to evaluate the cell viability of human T lymphocytes induced by co-stimulation of anti-CD3 mAb plus anti-CD28 mAb in response to PF. Data showed that PF effectively inhibited the cell viability of human T lymphocytes in a dose-dependent manner, relative to controls (Fig. 1b). Meanwhile, the cytotoxicity experiments were performed to eliminate the cytotoxic effect of the drug. The results showed that PF did not significantly influence the cell growth with the cell viability of higher than 80%, which covered the range of effective concentrations of PF used in the anti-proliferation experiments (data not shown).

Furthermore, we examined the effect of PF on the T lymphocytes apoptosis via Annexin V/PI staining. The results confirmed that PF at 50 μ M, 100 μ M and 200 μ M dosages significantly increased the percentage of apoptotic T lymphocytes in a dose-dependent manner (Fig. 1c). After stimulation with anti-CD3 mAb and anti-CD28 mAb for 72 h, the mean percentage of T lymphocytes apoptosis induced by 50 μ M PF, 100 μ M PF and 200 μ M PF was $(25.91 \pm 0.59)\%$, $(29.24 \pm 0.05)\%$ and $(34.47 \pm 1.10)\%$ respectively relative to the control group $(22.29 \pm 1.28)\%$ (Fig. 1c), indicating the growth inhibitory effects of PF on T lymphocytes were mediated by apoptosis.

3.2. PF inhibited the expressions of CD25, CD69 and NF- κ B p65 on activated T Lymphocytes

CD69 and CD25 represent cell surface markers at the early and late stages of T lymphocytes activation respectively [16,17]. NF- κ B signaling pathway plays an important role in T lymphocytes activation [18]. To further investigate how PF regulates the active marker CD25, CD69 and NF- κ B signaling pathway of T lymphocytes, the expression of CD25, CD69 and NF- κ B p65 was evaluated by flow cytometry. The results demonstrated that the percentage of CD25, CD69 and NF- κ B p65 on T lymphocytes were significantly increased after stimulated with anti-CD3 mAb and anti-CD28 mAb for 48 h compared with unstimulated conditions; 100 μ M and 200 μ M PF significantly inhibited CD25 of activated T lymphocytes ($P < 0.001$) (Fig. 2a); 100 μ M PF inhibited CD69 ($P < 0.05$), 200 μ M PF inhibited CD69 significantly ($P < 0.001$) (Fig. 2b); 50 μ M PF inhibited NF- κ B p65 of T lymphocytes ($P < 0.01$), 100 μ M and 200 μ M PF significantly inhibited NF- κ B p65 ($P < 0.001$) (Fig. 2c). The results showed that PF inhibited CD25, CD69 and NF- κ B p65 on T lymphocytes in a dose-dependent manner.

3.3. PF inhibited NF- κ B/p38 MAPK pathway and IFN- γ secretion in T lymphocytes

The effect of PF on IFN- γ and IL-4 production by T lymphocytes were examined. T lymphocytes were activated by anti-CD3 mAb and anti-

CD28 mAb for 72 h, and the culture supernatants were assayed for IFN- γ and IL-4 production. The result showed that there was greater than 50% inhibition of IFN- γ production at over 12.5 μ M of PF ($P < 0.001$) (Fig. 3a). However, there was no significant effect on IL-4 production ($P > 0.05$) (Fig. 3b).

NF- κ B pathway and MAPK pathway play important roles in the inflammatory reaction. To further study the mechanism of action of PF, we performed western blotting to detect the expression of related proteins in MAPK/NF- κ B pathway in T lymphocytes. The results (Fig. 3c) illustrated that compared with the control group, 10 μ M PF significantly inhibited the expression of NF- κ B p65 of activated T lymphocytes ($P < 0.05$); PF significantly inhibited the phosphorylation of I κ B α in a dose-dependent manner ($P < 0.01$). As shown in Fig. 3d, 5 μ M PF depressed p-p38 level ($P < 0.05$) and 10 μ M PF depressed p-p38 level significantly ($P < 0.01$) while the expression of p-ERK and p-JNK remained unchanged under PF exposure.

These results showed that PF depressed the activation of NF- κ B and p38 pathways and IFN- γ secretion in activated T lymphocytes.

3.4. PF inhibited NF- κ B pathway and IFN- γ secretion in T lymphocytes to attenuate the ACD response

To identify the mechanism of action of PF *in vivo*, we established DNCB-induced ACD mouse model. As shown in Fig. 4, administration of PF orally (75 or 150 mg/kg/day) significantly reduced mice ear swelling ($P < 0.01$) in a dose-dependent manner (Fig. 4a) and attenuated the inflammatory cell infiltration and epidermal keratinisation of ear skin lesions in comparison to ACD model group (Fig. 4b). What's more, oral administration of PF inhibited the proliferation of T lymphocytes in ACD mice in a dose-dependent manner (Fig. 4c).

Then, the secretion of IFN- γ and IL-4 by T lymphocytes were measured in different groups. The IFN- γ level was significantly higher in ACD model group than the control group ($P < 0.01$) and PF significantly reduced it in a dose-dependent manner ($P < 0.01$) (Fig. 5a). However, there was no significant change in IL-4 levels of different groups (Fig. 5b). In addition, the effect of PF on the NF- κ B/MAPK pathway in T lymphocytes was analysed by western blotting. As shown in Fig. 5c and Fig. 5d, compared with the control group, the expression of NF- κ B p65 was increased ($P < 0.05$) and the phosphorylation of I κ B α , p38, JNK, ERK were increased significantly ($P < 0.01$) in the DNCB-induced ACD model group. Meanwhile, administration of PF orally (75 or 150 mg/kg/day) significantly inhibited NF- κ B p65 and the phosphorylation of I κ B α in a dose-dependent manner ($P < 0.01$) (Fig. 5c) while had no significant effect on the MAPK pathway compared to ACD model group (Fig. 5d).

These results indicated that PF exerted the protective effects on DNCB-induced ACD through inhibiting the IFN- γ production and the NF- κ B/I κ B α pathway in T lymphocytes.

4. Discussion

PF has been proven to have a wide range of pharmacological effects, especially anti-inflammatory, analgesic and immunomodulatory effects. PF could exert such activities by regulating the proliferation or activation of immune cells such as T, B lymphocytes, macrophages, dendritic cells and vessel endothelial cells, as well as reducing the production of inflammatory cytokines and restoring abnormal signaling pathways [14,19]. In this study, we focused on the unique effects of PF on the activated T lymphocytes, which play a crucial role in skin sensitisation and allergic contact dermatitis [20], to explore how PF alleviates the T-cell-mediated ACD response. It is shown that PF depresses T lymphocytes activation via inhibiting IFN- γ production and NF- κ B signaling pathway, thereby exerting its anti-inflammatory and immunosuppressive effects in ACD.

Activation of T lymphocytes is primarily responsible for the immunopathogenesis of ACD and other skin diseases of the inflammatory

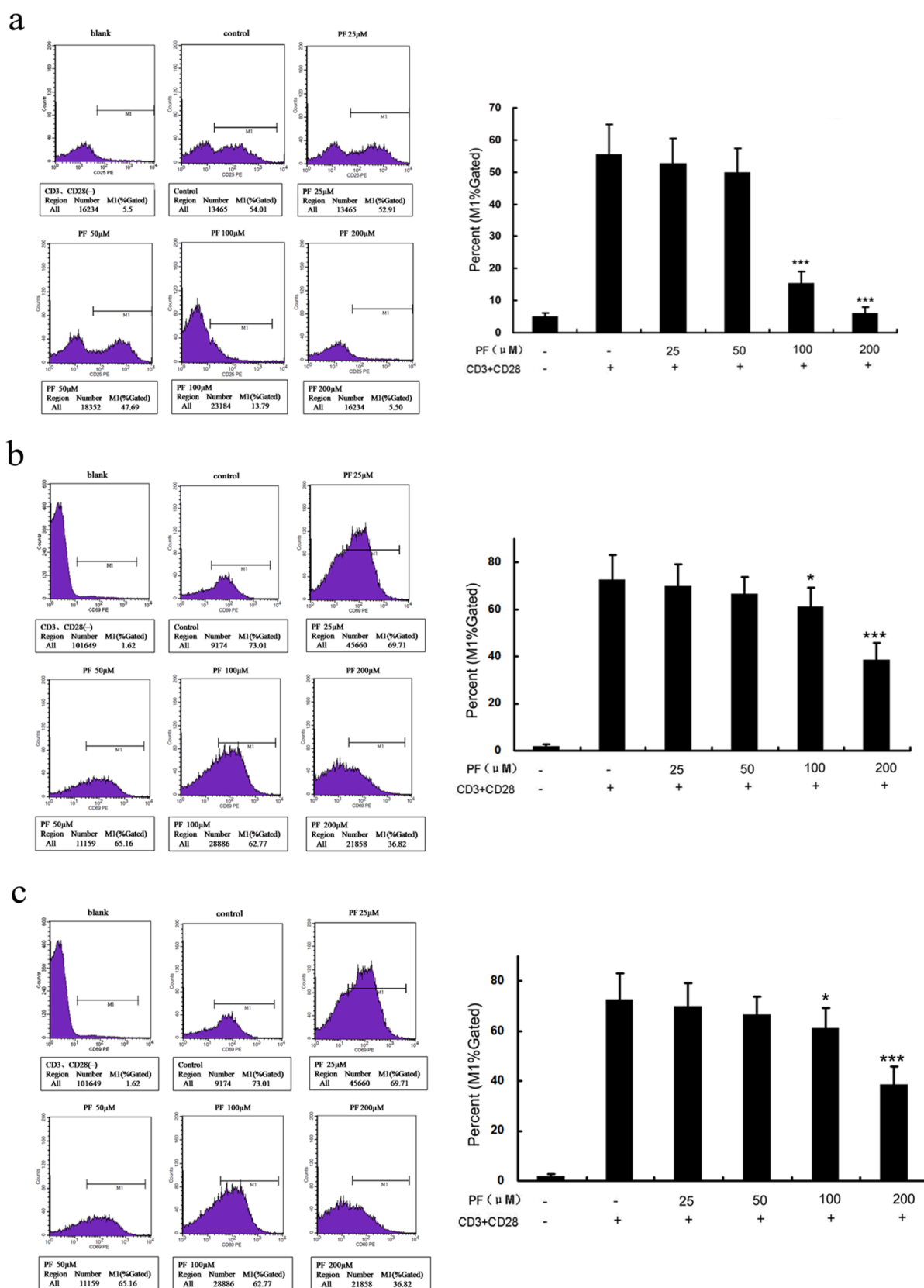


Fig. 2. Flow cytometry analysis of PF on CD25, CD69 and NF-κB p65 expression of human activated T lymphocytes. Activated T lymphocytes were cotreated with or without PF (25, 50, 100 and 200 μM) for 48 h and T lymphocytes without activation was blank. Analysis of PF on (a) CD25, (b) CD69 and (c) NF-κB p65 expression with the mean fluorescence intensity of each group. Error bars represent mean ± SD. * $P < 0.05$, *** $P < 0.001$, compared to activated cells without PF in control group, (n = 3).

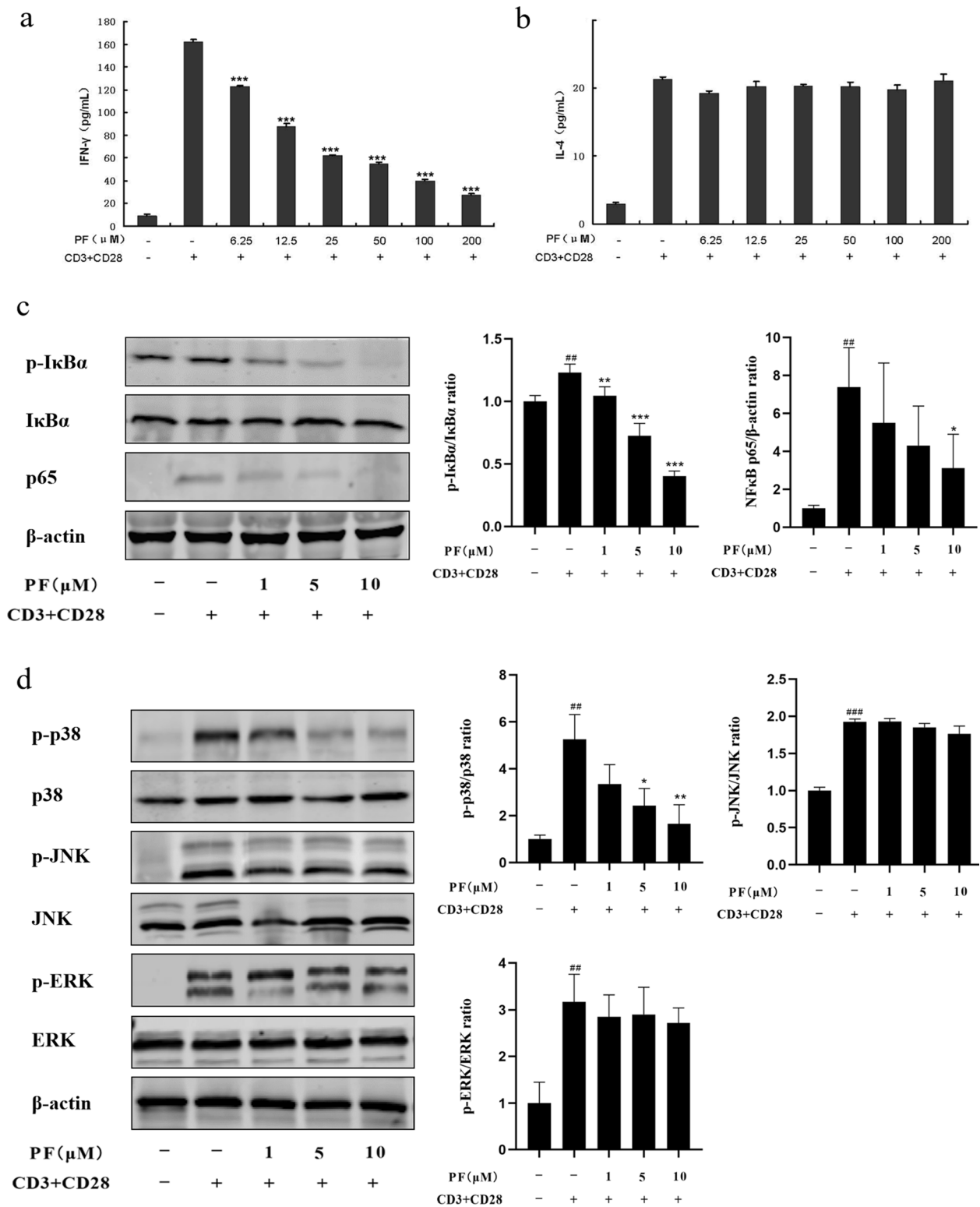


Fig. 3. Influence of PF on the cytokine levels and NF-κB/MAPK signaling pathways in human activated T lymphocytes. (a-b) The effect of PF on (a) the IFN-γ and (b) the IL-4 production. The culture supernatants of activated T lymphocytes were assayed for the concentration of IFN-γ and IL-4 by ELISA. Error bars represent mean ± SD. *** $P < 0.001$, compared to activated cells without PF in control group, (n = 3). (c-d) The effect of PF on (c) the NF-κB and (d) the MAPK signaling pathways activations. Error bars represent mean ± SD. ## $P < 0.01$, ### $P < 0.01$, compared to cells without both activated and PF in blank group; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared to activated cells without PF in control group, (n = 3).

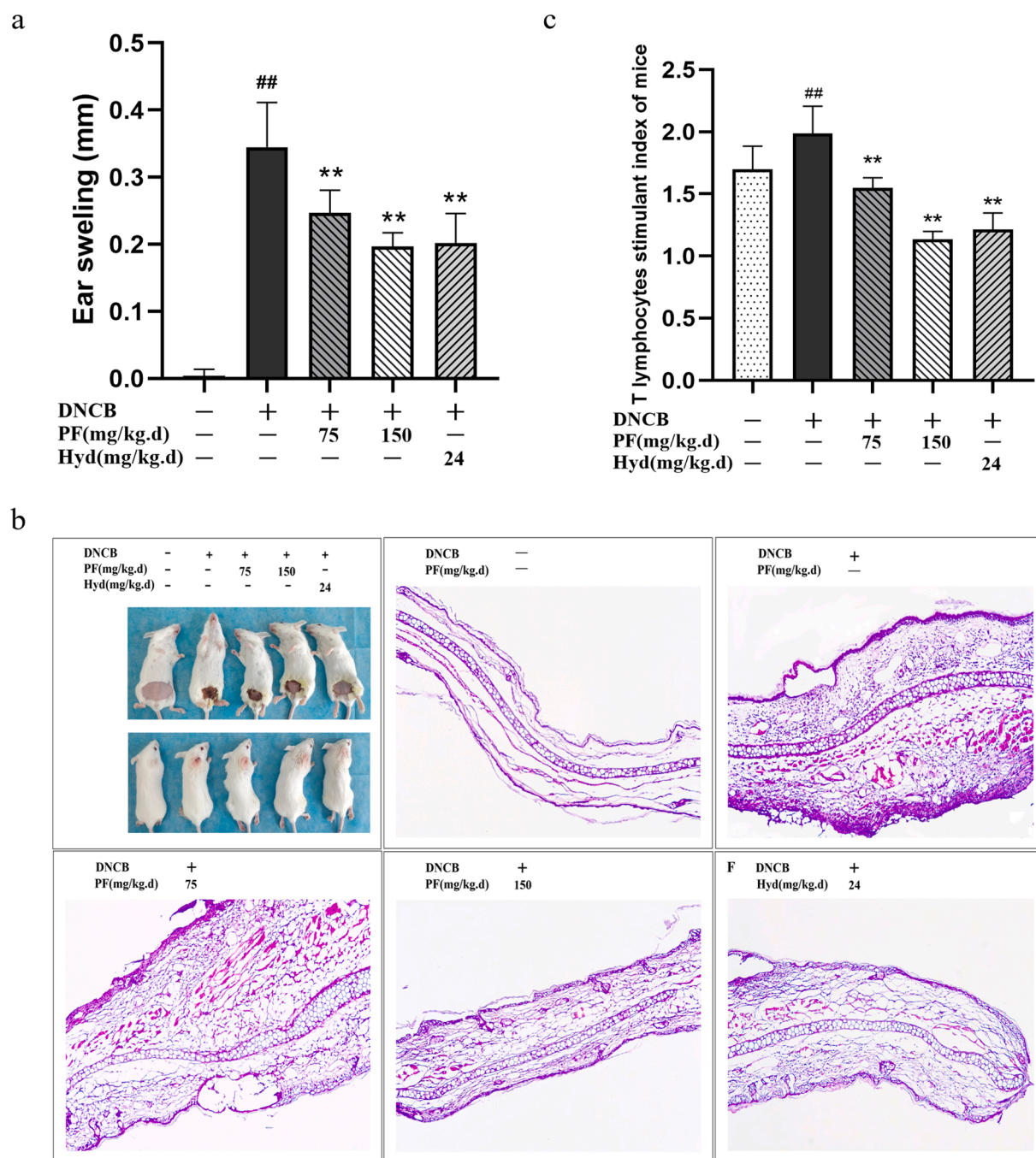


Fig. 4. The effect of PF administration on the ear swelling, histopathological features of ear skin lesions and T lymphocytes activation of mice with ACD. (a) Effects of PF on ear swelling and the thickness was measured by micrometer. (b) Histopathological changes of ear skin lesions in every group (original magnification, 100 $\times$). (c) Effects of PF on T lymphocytes in every group. Error bars represent mean $\pm$ SD. ^{##} $P < 0.01$, compared to normal group; ^{**} $P < 0.01$, compared to the model group control, (n = 8).

origin. T lymphocytes proliferation, one of the most important hallmarks of activation, as well as the apoptosis have significant impacts on the extent to which sensitisation is acquired. In our experiment, PF significantly suppressed the proliferation of activated human peripheral T lymphocytes activated by anti-CD3 mAb and anti-CD28 mAb as well as induced their apoptosis in a dose-dependent manner, which was also consistent with those of mouse T lymphocytes *in vitro* [21]. Furthermore, oral administration of PF also inhibited the proliferation of T lymphocytes in ACD mice. These data indicated that PF could depress the abnormal activation of T lymphocytes and exert an immunosuppressive effect.

Then, we interrogated the effect of PF on the expression of the

α -subunit of the IL-2 receptor (CD25), a common T lymphocytes activation marker. In agreement with several reports [17,22], CD25 was highly expressed in activated T cells. In the presence of PF, the up-regulation of CD25 was reduced significantly in a dose-dependent manner. The finding suggested that PF may suppress activated T lymphocytes proliferation through the inhibition of CD25 expression which, in turn, rendered the cells unresponsive to IL-2. CD69 is a type II C-type lectin receptor which is upregulated on T lymphocytes upon CD3/TCR-mediated activation [23]. Although known as a very early activation marker, CD69 was recently reported to exert costimulatory effects to influence T lymphocytes activation only under the ground of IFN- γ secretion in NK cells, CD25 upregulation in IL-2 treated T lymphocytes

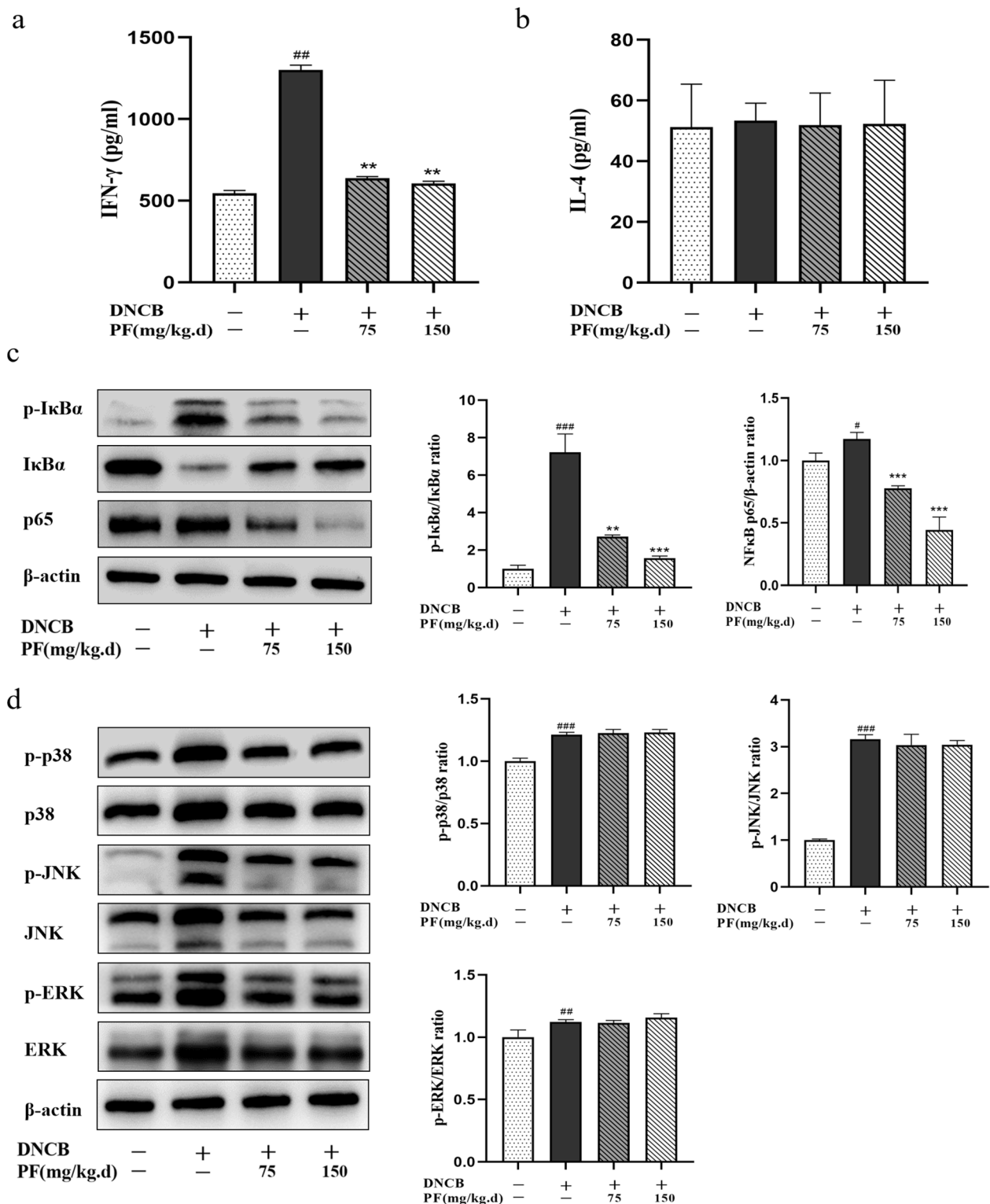


Fig. 5. Influence of PF on the cytokine levels and NF- κ B/MAPK signaling pathways in T lymphocytes of DNCB-induced ACD mice. (a-b) The effect of PF on (a) the IFN- γ and (b) the IL-4 production. Mice were sensitized and challenged with DNCB as previously described. Highly purified T cells were isolated and co-cultured with ConA for 48 h. The culture supernatants were assayed for the concentration of IFN- γ and IL-4 by ELISA. (n = 6). (c-d) The effect of PF on (c) the NF- κ B and (d) the MAPK signaling pathways activations. (n = 3). Error bars represent mean $\pm$ SD. [#] P < 0.05, ^{##} P < 0.01, ^{###} P < 0.001, compared to normal group; ^{**} P < 0.01, ^{***} P < 0.001, compared to the model group control.

and TGF- β secretion when crosslinked on anti-CD3 activated T lymphocytes [24]. In the current study, the increased expression of CD69 following anti-CD3 mAb and anti-CD28 mAb co-stimulation was seen and PF showed inhibiting effect on the upregulation of CD69 similar to

CD25. Additionally, we also found that PF significantly decreased IFN- γ (Th1) level in the activated T lymphocytes, but had no obvious effect on IL-4 (Th2) secretion. The suppression effect on both markers - CD25, CD63 - participating in T lymphocytes activation, together with the

inhibition of IFN- γ , suggested that PF might suppress T cell function at both late stage and early stage.

To further determine the underlying mechanism of the T lymphocyte activation-inhibiting effects of PF, the NF- κ B and MAPK signaling pathway have attracted attention. Previous studies have shown that ACD, psoriasis and atopic dermatitis could present abnormal expressions of NF- κ B and I κ B in skin lesions [3,25,26], suggesting NF- κ B/I κ B signaling pathway may be involved in the occurrence and development of these inflammatory skin diseases. In addition, the activation of MAPK, another classic inflammation-related pathway, could promote the production of inflammatory cytokines and aggravate the inflammatory response [27]. Meanwhile, it is reported that PF could reduce neuropathic pain, improve pancreatic β -cells injury and exert anti-inflammatory effects by regulating NF- κ B or MAPK pathways [28–30]. In this study, we found that PF inhibited the activation of NF- κ B/I κ B and p38 MAPK signaling pathways of human T cells *in vitro* while the effect of PF on phosphorylation of p-JNK and p-ERK was not significant, indicating PF may play immunosuppressive and anti-inflammatory effects as a potential NF- κ B targeted therapeutic agent.

What's more, Numerous pharmacokinetic studies have shown that paeoniflorin distributes rapidly and widely in various tissues of rats, mainly in the stomach (33.66 mg/mL) and intestine (19.57 mg/mL), but also in the heart, liver, spleen, brain, etc [19]. The hydrolysis of ester bonds and glucosidic bands, and the combination with glucuronides are the main metabolic pathways of PF [19]. Also, PF can be converted into metabolites including 7S-paeonimetalbolin I (7S-PM I) and 7R-PM I *in vivo* and *in vitro* [31,32]. After oral administration of PF, the main way of excretion is urine, and it is rarely excreted in the form of prototype drugs. However, the oral bioavailability of PF is only about 3%, which may be effectively improved by using glucosidase inhibitors to block efflux transporters or by structural modification such as 6'-acetylpaeniflorin [19,33]. The pharmacokinetic parameters including $t_{1/2\alpha}$, $t_{1/2\beta}$, AUC, MRT and $C_{\max}$ were increased significantly after oral administration of that, and performed a more effective therapeutic effect on ACD mice [33]. Additionally, effects of PF on ACD animal models were explained through various molecular mechanisms. Accumulating evidence shown that PF could regulate the unbalanced cytokine production and Shi et al. found that PF inhibited the migration of dendritic cells and the differentiation of Th1 and Th17 to attenuate ACD in the mouse model [34,35]. In the present study, as the IFN- γ expression and NF- κ B/I κ B α pathway activation of T lymphocytes in the ACD model were enhanced, we found that both of them were reduced in a dose-dependent manner under PF treatment. However, there was no effect on the IL-4 level and MAPK pathways activation. Anyway, the results suggest that PF could attenuate the DNCB-induced ACD response by inhibiting the production of IFN- γ and NF- κ B/I κ B α pathway in T lymphocytes.

5. Conclusion

To conclude, the immunosuppressive and anti-inflammatory effects of PF may be closely related to its inhibition of abnormal activation of T lymphocytes and involvement in the regulation of NF- κ B/I κ B α signaling pathways, which also means that PF may be a promising T cell-targeted drug candidate for ACD treatment. However, PF seems to have no significant effect on the phosphorylation of p38 in T lymphocytes compared with that of *in vitro*. Considering the complexity of cell networks, in-depth research is needed.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

Xiaoting Wu and Aoxue Wang designed the research, performed experiments and wrote the manuscript; Xiaoyi Qi, Jing Wang and Yunying Zhang performed experiments and interpreted data; Yanwei Xiao performed the statistical analyses; Caixia Tu and Aoxue Wang provided suggestions and reviewed. All authors approved the final manuscript.

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