

Effects of *Psoraleae fructus* and Its Major Component Psoralen on Th2 Response in Allergic Asthma

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Abstract: This study is aimed to evaluate the effects of *Psoraleae fructus* (PF) on Th2 responses in a rat model of asthma *in vivo* and psoralen, a major constituent in PF, on Th2 responses *in vitro*. A rat model of asthma was established by sensitization and challenged with ovalbumin (OVA). Airway hyperresponsiveness was detected by direct airway resistance analysis. Lung tissues were examined for cell infiltration and mucus hypersecretion. Bronchoalveolar lavage fluid (BALF) was assessed for cytokine levels. *In vitro* study, Th2 cytokine production was evaluated in the culture supernatant of D10.G4.1 (D10 cells) followed by the determination of cell viability, meanwhile Th2 transcription factor GATA-3 expression in D10 cells was also determined. The oral administration of PF significantly reduced airway hyperresponsiveness (AHR) to aerosolized methacholine and decreased IL-4 and IL-13 levels in the BALF. Histological studies showed that PF markedly inhibited inflammatory infiltration and mucus secretion in the lung tissues. *In vitro* study, psoralen significantly suppressed Th2 cytokines of IL-4, IL-5 and IL-13 by ConA-stimulated D10 cells without inhibitory effect on cell viability. Furthermore, GATA-3 protein expression was also markedly reduced by psoralen. This study demonstrated that PF exhibited inhibitory effects on hyperresponsiveness and airway inflammation in a rat model of asthma, which was associated with the suppression of Th2 response. Psoralen, a major constituent of PF, has

immunomodulatory properties on Th2 response *in vitro*, which indicated that psoralen might be a critical component of PF for its therapeutic effects.

Keywords: Asthma; Th2 Response; *Psoraleae fructus*; Psoralen.

Introduction

Allergic asthma, a chronic inflammatory lung disease of the airway, is characterized by airway inflammatory cells accumulation and goblet cell hyperplasia to inhaled allergens (Finkelman *et al.*, 2010; Bosnjak *et al.*, 2011). There is substantial evidence to indicate that T helper 2 (Th2) cells play a crucial role in the pathogenesis of asthma, and Th2 cell-derived cytokines are indicated to be the driving force behind airway hyperresponsiveness (AHR) development, inflammatory cells accumulation, and mucus hypersecretion (Wills-Karp *et al.*, 1998; Kips *et al.*, 2001). IL-4 and IL-13 are essential for IgE production and mucus hypersecretion, whereas IL-5 is critical in the determination of eosinophil recruitment, activation, and survival (Romagnani, 2000). GATA-3, a key transcription factor, has been regarded as not only critical for the development and differentiation of Th2 cells, but also faithful to the upregulation of Th2 cytokines (Hosoya *et al.*, 2010).

Psoraleae fructus (PF) (*Buguzhi* in Chinese), the seeds of *Psoralea corylifolia* L., is a commonly used traditional Chinese medicine, which has been widely used for the treatment of asthma, spermatorrhea, pollakiuria, and nephritis for thousand years in China (Pharmacopoeia Committee China, 2005). The major active constituents of PF include coumarins, flavonoids, and meroterpene phenols such as psoralen, isopsoralen, bovatichin (Lee *et al.*, 2012; Xiao *et al.*, 2012). Recently, PF and its constituents were discovered to have extensive pharmacological functions such as anti-inflammatory, antibacterial, antitumor, anti-oxidant, and hepatoprotective properties (Zhao and Xiao, 2006). Moreover, some studies have indicated that PF or its constituents can improve airway inflammation in the asthma model (Yu *et al.*, 2006; Hu *et al.*, 2008). Nevertheless, the mechanisms through which PF exerts its antiasthmatic effects have not been fully elucidated. And there has been no previous report of the effects of psoralen, one of the major constituents of PF, on Th2 responses, the key pathological mechanisms of asthma.

Considering the reported anti-inflammatory effects of PF on asthma, we hypothesized that this product may have anti-Th2 inflammatory effects *in vivo*. In order to test this possibility, we evaluated the antiasthmatic properties of PF in a rat model of asthma. As it is meaningful to investigate the therapeutic effects of the critical components in PF, we then tested the effects of psoralen, a major constituent of PF, on Th2 responses *in vitro* by using the classical Th2 clone, D10.G.4.1 (D10) cells.

Materials and Methods

Reagents

Psoralea fructus (PF) was purchased from Shanghai KangQiao Herbal Co. (Shanghai, China), and its extract was prepared as follows: 1.2 kg dried PF was soaked in 7.2 L water

for 2 hours and extracted twice with H₂O at 100°C. After filtration, the decoction was diluted in distilled water to a concentration of 1 g/ml (each ml of solution contained 1 g crude drug of PF). Psoralen was obtained from Shanghai Ronghe Co (Shanghai, China). Ovalbumin (OVA), aluminum hydroxide, and methacholine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dexamethasone sodium phosphate injection (5 mg/ml) was provided by Xianju Medicine Co. (Zhejiang, China). ELISA kits for IL-4, IL-5, IL-13 and IL-10 were supplied from Xitang Biological Pharmaceutical Co. (Shanghai, China), and INF- γ was provided by BioLegend Inc. (San Diego, USA). Polyclonal rabbit antibodies against GATA-3, β -actin and horseradish peroxidase (HRP)-conjugated goat anti-rabbit or mouse IgG were purchased from Beyotime Institute of Biotechnology (Jiangsu, China).

Animals

Male specific-pathogen-free Sprague–Dawley (SD) rats weighing 200–250 g were purchased from Shanghai SLAC Co. (Shanghai, China). Animals were housed in stainless steel cages in a temperature-controlled environment ($22 \pm 2^\circ\text{C}$) on a 12-h light-dark schedule (lights on from 6:00 AM to 6:00 PM) and they were given free access to water and rodent chow. Those animals were acclimatized for at least 7 days before use in experiments. Sixty rats were randomly divided into six groups as follows: a control group (10 rats), an OVA control group (10 rats), a DEX (0.5 mg/kg) treatment group (10 rats), and three PF (2.5, 5.0, or 10.0 g/kg) treatment groups (10 rats per group). The animal study protocols have been approved by the Institutional Animal Care and Use Committee of Fudan University.

Sensitization and Treatment

As previously described, a rat model of asthma was developed by OVA sensitization and inhalation (Jin *et al.*, 2013). Briefly, on day 0, rats were systemically sensitized with an intraperitoneal injection of 1 ml alum-precipitated Ag in PBS which contained 100 mg of OVA mixed with 100 mg aluminum hydroxide. Two weeks after the sensitization, the rats were exposed to aerosolized OVA for 30 min/day for 4 weeks. The concentration of OVA was increased from 1% for 3 weeks to 2% for 1 week to maintain the asthma condition. From day 14 to day 42, the OVA-sensitized rats in the treatment groups were given PF or DEX orally once per day 1 hour before OVA inhalation, at doses of 2.5, 5.0, and 10.0 g/kg/day for PF and 0.5 mg/kg/day for DEX. The rats in the normal group received the same volume of PBS instead. The protocol for the sensitization, challenge and drug administration is summarized in Fig. 1.

Measurement of AHR

AHR was evaluated by a Buxco's modular and invasive system (Buxco Electronics Inc., NY, USA). Changes in airway resistance (R_L) and lung dynamic compliance (C_{dyn}) were measured directly as described by Amdur and Mead (Pichavant *et al.*, 2007). Briefly, each

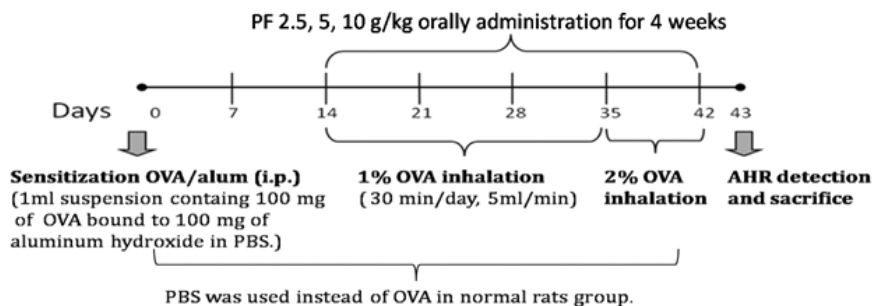


Figure 1. Experimental protocol. Rats were immunized with intraperitoneal injection of a suspension containing 100 mg OVA and 100 mg aluminum hydroxide. Two weeks after the immunization, rats were challenged by exposure to an aerosol of 1% OVA for 3 weeks and followed by 2% OVA for 1 week to maintain the asthma condition. From days 14 to 42, rats in the treatment groups were orally given PF at 2.5, 5.0, and 10.0 g/kg/day or DEX at 0.5 mg/kg/day. The rats in the normal group received the same volume of PBS instead.

anesthetized rat was tracheostomized and intubated with an appropriate cannula, and then laid supine inside the body plethysmograph chamber connected to the ventilator. After a stable baseline airway pressure ($<5\%$ variation over 2.5 min) was achieved, the rats were given aerosolized PBS or various concentrations of methacholine (3.125, 6.25, 12.5, or 25 mg/ml) via a jet nebulizer into the head chamber. Minimum values for R_L and C_{dyn} were calculated in response to increasing concentrations of inhaled methacholine and expressed as a percent change from the baseline value, as in previous studies (Glaab *et al.*, 2001).

Histological Analysis

After BALF was isolated, lung tissue slices were inflated with 10% neutral-buffered formalin. Thin sections (3–4 μm) were cut from blocks and stained with hematoxylin-eosin (H&E), periodic acid Schiff (PAS). Image-Pro Plus software was used for histopathological analysis. The severity of inflammatory cell infiltration in the lung was evaluated with a 5 point scoring system: 0, no cells; 1, a few cells; 2, a ring of cells 1 cell layer deep; 3, a ring of cells 2–4 cells deep; 4, a ring of cells >4 cells deep. PAS staining was used to evaluate the levels of airway mucus expression; at least 6 bronchioles were counted in each slide. The results were expressed as the area of airway epithelium staining positive for mucus/the perimeter of the basement membrane of the bronchioles.

Preparation and Analysis of Bronchoalveolar Lavage Fluids (BALFs)

Immediately followed by the assessment of AHR, BALF was obtained by inserting a tracheal tube and lavaging the lung two times with 4 ml sterilized normal saline containing 2% bovine serum albumin (BSA). The collected lavage fluid was centrifuged at $500 \times g$ at 4°C for 10 min. The supernatants were harvested and stored at -80°C for measurements of cytokine production. Interleukin (INF- γ , IL-4, IL-5, IL-13 and IL-10) levels in the BALF supernatant were analyzed by ELISA according to the manufacturer's instructions.

Cell Culture

The Th2 clone D10.G4.1 (D10) cell line was obtained from American Type Culture Collection (ATCC; Manassas, VA) and was cultured according to ATCC recommendations. Briefly, cells were grown in RPMI 1640 medium with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, and 1.0 mM sodium pyruvate, and supplemented with: 0.05 mM 2-ME, 10 pg/ml IL-1 α , 10% FBS, and 10% rat T-STIM factor with Con A (rat IL-2 culture supplement available from BD Biosciences, cat. no. 354115). The medium was changed every three days.

Determination of Cell Viability

Cell viability was determined by Cell counting kit-8 assay. In brief, D10 cells were placed in a 96-well plate at a concentration of 10,000 cells per well. D10 cells were treated with vehicle or psoralen (2, 4, and 8×10^{-5} M) for 48 h, or pretreated with vehicle or psoralen (2, 4, and 8×10^{-5} M) for 2 h, and then they were stimulated with ConA (50 μ g/ml) in presence or absence of psoralen or dexamethasone. After 48 h, the cells were then added with 10 μ l CCK8 reagents and incubated for 3 h at 37°C. The absorbance values were measured at 450 nm by using a Quant MQX200 microplate reader (Biotek Instruments, Winooski, VT, USA). The viability of D10 cells in each well was presented as percentage of the cells in medium only or treated with ConA.

Measurement of Th2 Cytokines in Cell Culture Supernatant

D10 cells were plated in a 24-well plate at a concentration of 10^5 cells per well. And then cells were pretreated with vehicle or psoralen (2, 4, and 8×10^{-2} mM) for 2 h. After pretreatment, cells were stimulated with ConA (50 μ g/ml) in presence or absence of psoralen or dexamethasone. After 48 h supernatants were collected and IL-4, IL-5, IL-13 and IL-10 levels were determined by ELISA according to the manufacturer's instructions.

Western Blot Analysis Assay of GATA-3 Expression in D10 Cells

GATA-3 expression was determined by Western blot analysis using a polyclonal anti-GATA-3 antibody stated above. D10 cells were incubated in a 6-well plate at a concentration of 10^6 cells per well. And then cells were pretreated with vehicle or psoralen (2, 4, and 8×10^{-5} M) for 2 h. After pretreatment, cells were stimulated with ConA (50 μ g/ml) in the presence or absence of psoralen or dexamethasone for 48 h. Cells were then collected and lysed. After denaturation at 95°C for 5 min, 30 μ g of total proteins was separated by 10% SDS-PAGE and transferred onto a nitrocellulose membrane. After blotting, the membranes were incubated with anti-GATA-3 antibody and β -actin, respectively. After washing three times to remove the primary antibody, HRP-conjugated secondary antibodies were applied, and the blots were developed by using enhanced chemiluminescence (ECL) reagents. The proteins were detected by using imagequant las4000 ECL SYSTEM.

Statistics

Statistical analysis was carried out by using SPSS 16 software (SPSS Inc., Chicago, IL, USA). Data was expressed as mean $\pm$ standard error (SEM). The significance of the differences between groups was determined by one-way ANOVA followed by post hoc Dunnett's tests. *p* values less than 0.05 were considered to indicate significant differences from the OVA control.

Results

Effect of PF on Enhanced AHR in Response to Methacholine

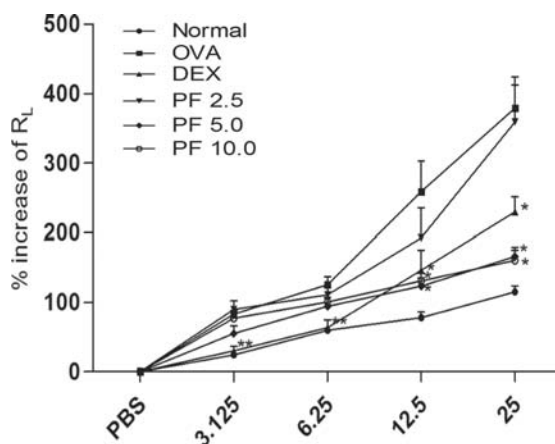
To detect the effects of PF on OVA-induced AHR, the airway responsiveness to aerosolized PBS or increasing concentrations of methacholine was assessed within 24 hours after the final challenge. Only mild changes in airway resistance (R_L) and lung dynamic compliance (C_{dyn}) were observed in normal rats. However, the R_L induced by methacholine at each level of dose ranging from 3.125 to 25 mg/ml was significantly increased, while the C_{dyn} was obviously decreased in the OVA group compared with that in the control group (Fig. 2). Oral administration of PF at dose of 10.0 g/kg significantly reduced the R_L and improved the C_{dyn} . Treatment with DEX also showed a similar inhibitory effect on the reduction of AHR compared with the high-dose of PF (Fig. 2).

Effect of PF on Inflammatory Cells, and Mucus Production in Lung Tissues

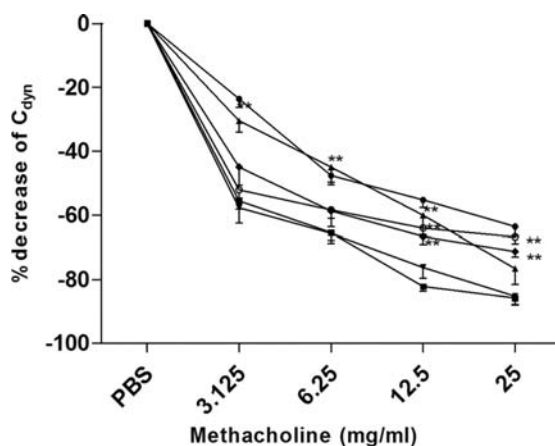
To demonstrate the inhibitory effects of PF on inflammation in the lung tissue, pulmonary pathology was observed by H&E staining. As shown in Fig. 3, a significant infiltration of inflammatory cells into peribronchiolar and perivascular connective tissues was observed in OVA-exposed rats. In contrast, PF treatment remarkably attenuated airway inflammation at the dose of 10.0 g/kg. PAS staining was conducted to evaluate the effect of PF on mucus production in lung tissues. The OVA-exposed rats exhibited a marked increase of mucus hypersecretion within the bronchi in the lungs. In contrast, the rats in the 10.0 g/kg PF treatment groups exhibited significantly reduced mucus production, similar to those found in the DEX group.

Effect of PF on Th1/Th2 Cytokines in BALF

To evaluate the effect of PF on the pulmonary immune response in allergic rat, the concentration of IL-4, IL-5, IL-13, IL-10 and IFN- γ in BALF were measured by ELISA. As shown in Fig. 4, OVA-exposed rats presented significantly elevated levels of IL-4, IL-5, IL-13 (but not IL-10), as well as reduced IFN- γ in the BALF in comparison to normal rats. Though there was no effect on elevated levels of IL-5 and IL-10, the oral administration of PF at the dose of 10 g/kg obviously inhibited the up-regulation of IL-4 and IL-13 levels. The effect of PF at 10 g/kg was comparable to that of DEX at 0.5 mg/kg. However, IFN- γ level



(A)



(B)

Figure 2. Airway responsiveness to aerosolized methacholine was evaluated by a Buxco's modular and invasive system, as described in the Materials and Methods. The rats were laid in a supine position inside the body plethysmograph chamber and were nebulized with PBS followed by increasing doses (3.125 to 25 mg/ml) of methacholine. Data are expressed as mean $\pm$ SEM. * p < 0.05, ** p < 0.01 versus the OVA group.

in the three PF treatment groups and DEX group was not significantly different from the OVA group.

Psoralen Treatment Showed no Inhibitory Effect on D10 Cells In Vitro

To determine the role of psoralen, an active ingredient of PF, on Th2 response *in vitro*, firstly, the effect of psoralen on D10 cell viability was evaluated by CCK-8 assay. Results showed that the absorbance values of D10 cells were significantly reduced in the presence of DEX. However, psoralen incubation alone for 48 h at concentrations of 2, 4, and

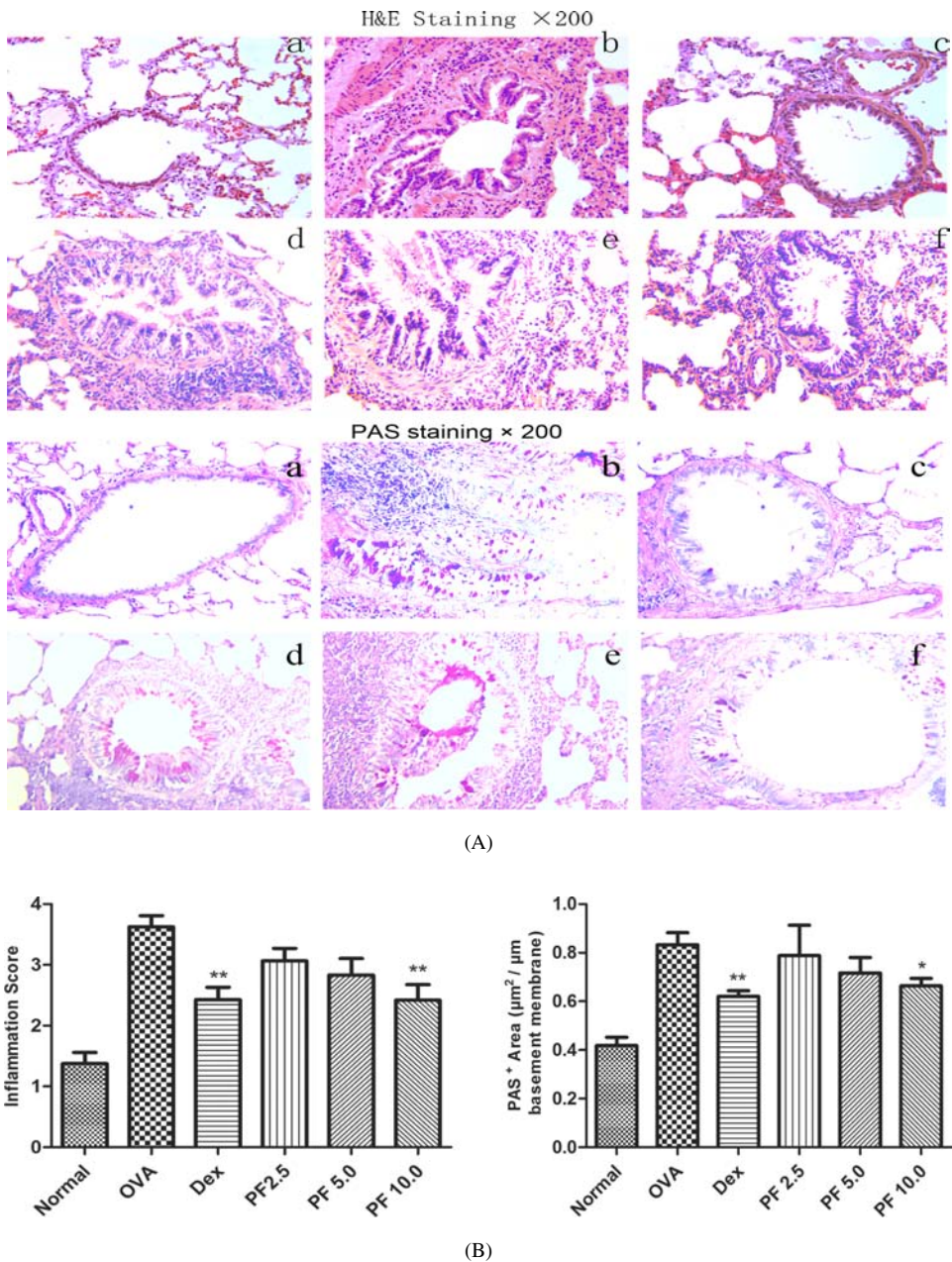


Figure 3. The effect of PF on airway inflammation and mucus production in the lung tissue. (A) Lung tissue slices were fixed, embedded, sectioned at 3–4 μm and stained with H&E and periodic acid-Schiff (PAS). (B) Graphs represent the inflammation score, PAS-positive area in the bronchial basement membrane. The results are expressed as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$ versus the OVA group. Representative pictures are shown for each group: a: normal; b: OVA; c: DEX; d: PF 2.5 g/kg group; e: PF 5.0 g/kg group; f: PF 10.0 g/kg group.

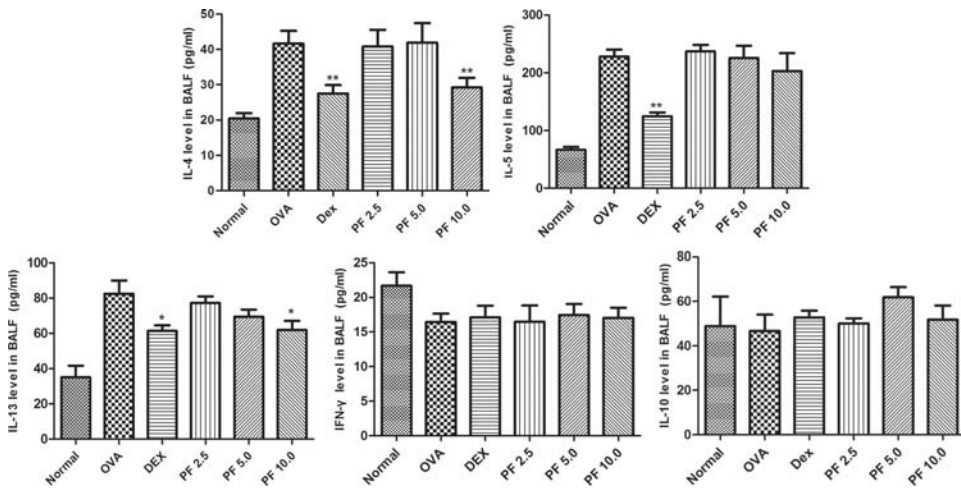


Figure 4. The effect of PF on cytokine secretion in BALF in a rat model of asthma. Secreted levels of the cytokines INF- γ , IL-4, IL-5, and IL-13 were measured by ELISA. Data are expressed as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$ versus the OVA group.

8×10^{-2} mM had no effect on the viability of D10 cells (Fig. 5A). Additionally, when cells were pretreated with psoralen (2, 4, and 8×10^{-2} mM) for 2 h before exposure to ConA ($50 \mu\text{g/ml}$) for 48 h, no significant difference in cell viability was found in comparison with the ConA-treated group (Fig. 5B), while ConA obviously promoted the proliferation of D10 cells, and DEX inhibited the proliferation of D10 cells. Thus, for this study, we used psoralen at concentrations of $2 - 8 \times 10^{-2}$ mM, which caused no cytotoxicity in the following experiments of anti-inflammatory property.

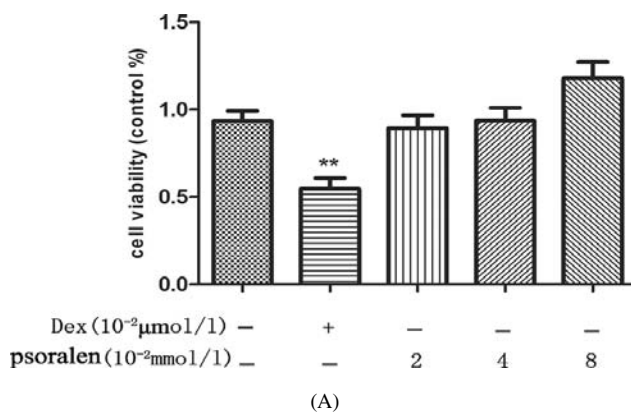


Figure 5. The effect of psoralen and/or ConA treatment on D10 cell viability. (A) Cells were treated with vehicle or psoralen ($2 - 8 \times 10^{-2}$ mM) for 48 h. (B) Cells were pretreated with vehicle or psoralen ($2 - 8 \times 10^{-2}$ mM) for 2 h and then exposed to ConA ($50 \mu\text{g/ml}$) for 48 h. After treatment, cell viability was determined by CCK-8 analysis as described in the Materials and Methods. Data are expressed as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$ versus the blank or ConA group.

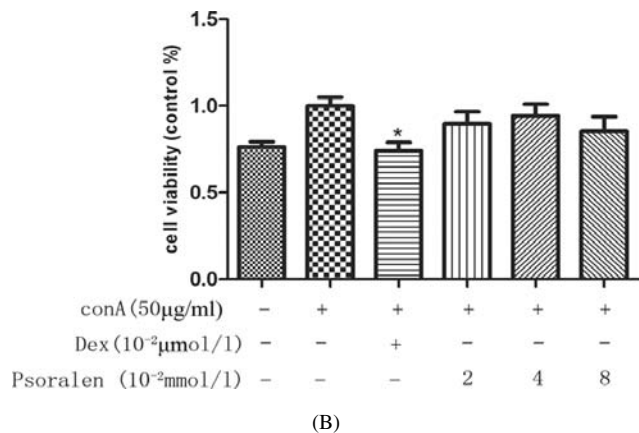


Figure 5. (Continued)

Psoralen Inhibited ConA-Induced Enhancement of Th2 Cytokines In Vitro

To determine whether psoralen suppressed Th2 cytokine synthesis, IL-4, IL-5, IL-13 and IL-10 levels in D10 cell culture supernatants following treatment with psoralen were determined by ELISA. As shown in Fig. 6, ConA-stimulated IL-4, IL-5, IL-13 and IL-10

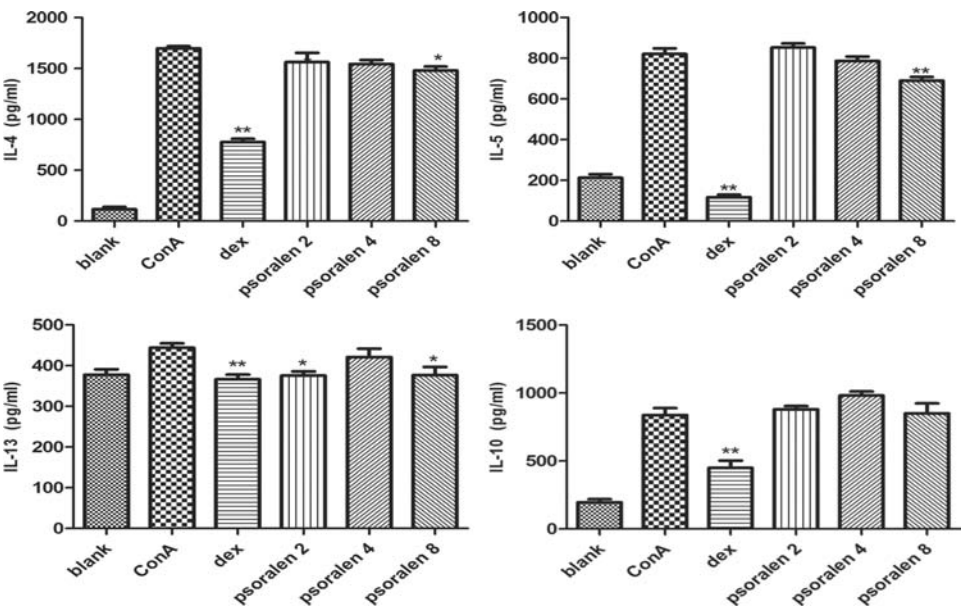


Figure 6. IL-4, IL-5, and IL-13 in culture supernatants of D10 cells treated with psoralen. Cells were pretreated with psoralen ($2-8 \times 10^{-2}$ mM) for 2 h and then exposed to ConA ($50 \mu\text{g/ml}$) for 48 h. After treatment, supernatants were collected and analyzed by ELISA for IL-4, IL-5, and IL-13. Data are expressed as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$ versus the ConA group.

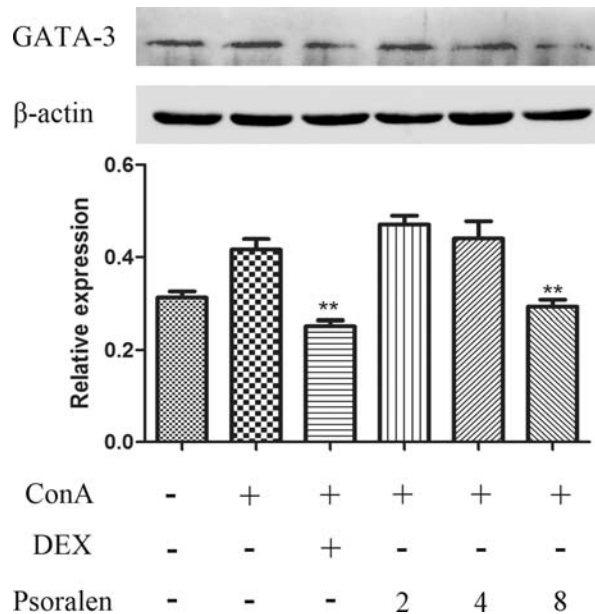


Figure 7. Western blot of GATA-3 in D10 cells in the presence of different concentrations of psoralen. GATA-3 expression was determined using polyclonal antibodies specific for GATA-3. Data are expressed as mean $\pm$ SEM. ** $p < 0.01$ versus the ConA group.

production were significantly reduced in the presence of DEX. Psoralen at 8×10^{-2} mM markedly suppressed the up-regulation of IL-4 IL-5 and IL-13, while psoralen at 2×10^{-2} mM obviously inhibited the levels of IL-13, but with no negative or positive impact on IL-10.

Psoralen Suppressed Memory Th2 Cell Transcription Factor GATA-3 Expression In Vitro

To determine whether the effects of psoralen on type 2 cytokines were transcriptionally regulated, Th2 transcription factor GATA-3 was measured by western blot. D10 cells were stimulated with ConA in the presence and absence of concentrations of psoralen ranging from 2 to 8×10^{-2} mM. As shown in Fig. 7, GATA-3 protein expression was markedly suppressed by psoralen at 8×10^{-2} mM, the effect of which was comparable to that of DEX at 0.5 mg/kg.

Discussion

In the present study, a rat model of asthma was employed to assess the effect of PF on the inhibition of Th2 response. We found the oral administration of PF markedly suppressed increased AHR in allergic rat. Allergic inflammatory infiltration and mucus secretion in the lung tissues were significantly reduced by PF administration. Besides, PF also inhibited the

OVA-induced elevations of IL-4 and IL-13 in BALF. Psoralen is a major constituent of PF, thus we tested the effect of psoralen on effector memory Th2 cells *in vitro*. We showed that psoralen dramatically suppressed the secretion of IL-4, IL-5 and IL-13 in the D10 cell culture supernatants. Th2 transcription factor GATA-3 was also significantly inhibited by psoralen. Collectively, all of these results demonstrated that PF and psoralen could significantly suppress Th2 response *in vivo* and *in vitro* respectively.

Airway hyperreactivity is one of striking features of allergic asthma, which is characterized by episodic bronchoconstriction and obstruction in response to a provoking stimulus. In this study, AHR was accurately assessed by an invasive measurement with determining changes in pulmonary resistance (R_L) and dynamic compliance (C_{dyn}). Our results demonstrated a dramatic reduction of R_L and improvement of C_{dyn} in PF-treated rats at 10 g/kg compared with untreated controls. Th2 cytokines are widely reported to be involved in the induction of AHR (Bosnjak *et al.*, 2011). IL-4, IL-5, and IL-13 have been demonstrated to play a critical role in the development of allergen-induced AHR (Webb *et al.*, 2000; Walter *et al.*, 2001). It was found that PF significantly inhibited and enhanced levels of IL-4 and IL-13 in the BALF at 10 g/kg, which may be associated with the inhibitory effects of PF on AHR.

Airway inflammation with mucus hypersecretion is another characteristic feature of asthma. In the present study, it was found PF significantly inhibited inflammatory cell infiltration and mucus hypersecretion by histological analysis. Th2 cytokines played a key role in promoting inflammation in lungs and mucus hypersecretion by epithelial goblet cells (Busse and Lemanske, 2001). Thus, the observed reduction of Th2 cytokines of IL-4 and IL-13 may contribute to the inhibitory effects of PF on airway inflammation and mucus hypersecretion.

In the present study, we observed that treatment with PF did not cause any impact in the levels of IL-10 and INF- γ in BALF of OVA-challenged rat, indicating that the suppressive effect of PF on Th2 response *in vivo* was not associated with the production of the anti-inflammatory cytokines or to a shift toward a Th1 prolife.

As explained here above, Th2 cells hold a key position in the orchestration of airway inflammation in asthma. In the current study, it was demonstrated that PF has inhibitory effects on Th2 response *in vivo*. However, it was not convenient to examine the effect of PF on Th2 response *in vitro* as PF includes complex constituents. Thus, we examined whether and how psoralen, one of major constituents of PF, affected Th2 response *in vitro* by using the classical Th2 clone, D10 cells. Our *in vitro* study showed that psoralen at 8×10^{-2} mM significantly suppressed production of Th2 cytokines, IL-4, IL-5, and IL-13 by ConA-stimulated D10 Cells, with no positive impact on IL-10. It suggested that the inhibitory effect of psoralen on Th2 response *in vitro* did not seem to be dependent on the production of the anti-inflammatory cytokine. Incubation of D10 cells with psoralen ($2-8 \times 10^{-2}$ mM) for 48 h showed no significant cytotoxic effect on cell viability, implying that down-regulation of Th2 cytokines by psoralen treatment was not due to decrease in cell numbers.

GATA-3 is a critical transcription factor for Th2 cell differentiation and is essential for the gene expression of the cytokines (Barnes, 2008). It was suggested that GATA-3 could

directly activate the transcription of the IL-4 gene through this 800-bp promoter region (Zheng and Flavell, 1997). Furthermore, GATA-3 was proposed to be directly involved in the initiation of transcription machinery on the IL-5 and IL-13 promoter, and therefore it is indispensable for IL-5 and IL-13 production in Th2 cells (Kishikawa *et al.*, 2001; Klein-Hessling *et al.*, 2003). In this study, we demonstrated that psoralen at 8×10^{-2} mM significantly suppressed GATA-3 protein expression by ConA-stimulated D10 cells. Thus, down-regulation of GATA-3 expression in D10 cells might, at least in part, account for suppression of Th2 response *in vitro* by psoralen.

In conclusion, the present study demonstrated that PF could effectively attenuate AHR and airway inflammation, which might depend on the suppression of Th2 cytokines *in vivo*. Our *in vitro* results found that psoralen significantly reduced production of Th2 cytokines and GATA-3 expression in ConA-stimulated D10 cells. These findings supported the theory that PF was a potent agent for inhibition of Th2 responses in asthma, and psoralen might be a critical component in PF for its therapeutic effects. However, further investigation is still required to clarify the effect of psoralen on asthma *in vivo* study.

Acknowledgments

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