



Regulatory effect of baicalin on the imbalance of Th17/Treg responses in mice with allergic asthma



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ABSTRACT

Ethnopharmacology relevance: Baicalin, a flavonoid compound, was isolated from traditional Chinese medicine *Scutellaria baicalensis* Georgi. The study aimed to explore the regulatory effect of baicalin on immunological balance of Th17/Treg responses and the possible mechanisms in mice with allergic asthma.

Materials and methods: Mice were sensitized and challenged with OVA+LPS by intranasal instillation, and were intragastrically treated with baicalin from days 22–36 after sensitization. The organ coefficient of lung was determined. Immunoglobulin E (IgE) level in serum and cytokine IL-17A, IL-6, IL-10 levels in bronchoalveolar lavage fluid (BALF) were measured by ELISA. Histological changes in lung and airway tissues were observed by hematoxylin and eosin (H & E) and periodic acid-Schiff staining (PAS). The expressions of signal transducer and activator of transcription 3 (STAT3) and forkhead box P3 (FOXP3) in lung tissues were evaluated by immunohistochemistry and western blot methods.

Results: Baicalin obviously decreased OVA+LPS-induced organ coefficient of lung, inhibited serum IgE and BALF IL-7A and IL-6 secretion, promoted BALF IL-10 secretion in a dose-dependent manner. Histological studies demonstrated that baicalin significantly alleviated OVA+LPS-induced inflammatory responses and mucus secretion in lung and airway tissues. Immunohistochemistry and western blot studies showed that baicalin substantially suppressed STAT3 expression and promoted FOXP3 expression in lung tissues of mice.

Conclusions: These findings suggest that baicalin effectively protects against OVA+LPS-induced allergic asthma in mice by regulating the immunological imbalance of Th17/Treg responses.

1. Introduction

Allergic asthma is a chronic airway inflammatory response, which involved multiple types of cells, cytokines, chemokines and transcription factors. It is mainly manifested by a large number of inflammatory cell infiltration, inflammatory cytokine release, mucus hypersecretion, bronchial spasm, airway hyperresponsiveness (AHR) and other symptoms (Kay, 1991; Bousquet et al., 2000). It has always been thought that the majority of asthma is associated with immune-mediated imbalance response between Th1 and Th2 (Wegmann, 2009; Bosnjak et al., 2011). Recent studies showed that asthma is complex in pathogenesis and also associated with immune-mediated imbalance response between Th17 and Treg (Cosmi et al., 2011). Corticosteroids, antihistamines and leukotriene receptor antagonists are used as the main therapeutic drugs for asthma. However, these treatments for asthma are not curative, and symptoms return soon after treatment termination (Akdis, 2012; Manson et al., 2009; Kleiman and Tuckermann, 2007). Thus, the development of efficient therapeutic

drugs for allergic asthma disease is urgently needed.

Traditional Chinese medicines are a rich source of new therapeutic drugs for allergic asthma. *Scutellaria baicalensis* Georgi has been widely used in traditional Chinese medicines. Baicalin (Fig. 1) is a major flavonoid compound isolated from the dried roots of *Scutellaria baicalensis* Georgi. It has been shown that baicalin has various pharmacological activities, such as antioxidant, antibacterial, antitumor, anti-inflammatory, antiallergic effects (Yang et al., 2015). Based on its anti-inflammatory activity, baicalin is widely used to treat various inflammatory diseases including hepatitis, atopic dermatitis, bronchitis and asthma (Chen et al., 2001), particularly, respiratory inflammatory diseases. Some recent studies have reported its anti-asthma effects. It was reported that baicalin inhibited OVA-induced asthmatic airway remodeling in asthmatic mice by decreasing expression of transforming growth factor- β 1, IL-13 and vascular endothelial growth factor (Sun et al., 2013). Another study showed baicalin has anti-asthmatic effects on asthma in OVA-induced mice by regulating Th1/Th2 (Ma et al., 2014) and inhibits PDGF-induced proliferation

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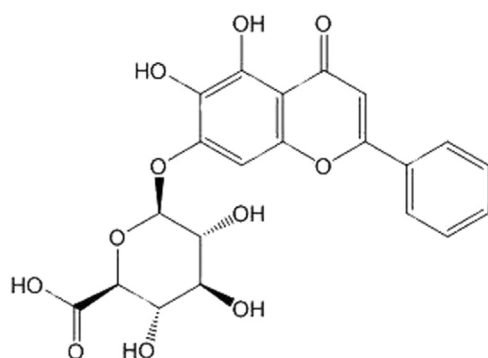


Fig. 1. Chemical structure of baicalin.

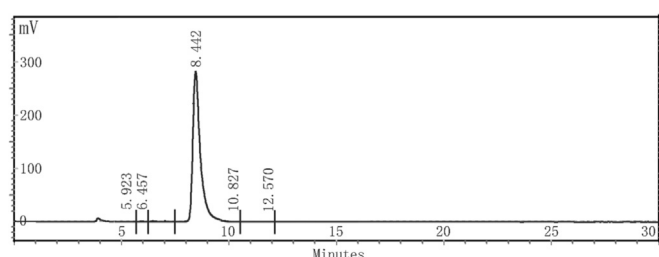


Fig. 2. HPLC profile of baicalin. The profile of baicalin was achieved on SinoChrom ODS-BP 5 μ m column. The mobile phase was consisted of methanol-0.1% phosphoric acid (60:40, v/v) and the flow rate was 0.8 ml/min.

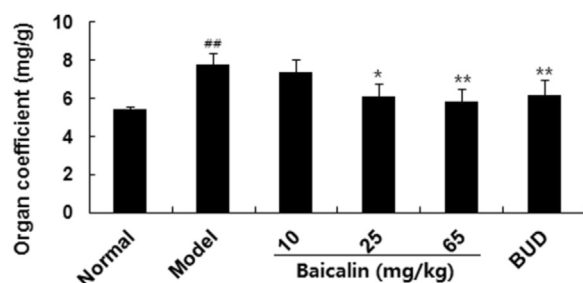


Fig. 3. Effects of baicalin on organ coefficient of lung tissues in OVA+LPS-induced asthmatic mice. Mice were sensitized and challenged with OVA combined with LPS, and intragastrically treated with baicalin (at doses of 10, 25 and 65 mg/kg). The lung tissues were removed and weighed, the organ coefficient of lung tissues were calculated. The data were expressed as means $\pm$ SEMs. $^{**}P < 0.01$ vs. normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. model group.

and migration of airway smooth muscle cells (Yang et al., 2015). However, no study thus far has addressed whether baicalin has protective effect on OVA+LPS-induced asthma and the possible involved mechanisms. In this study, we aimed to explore the potential protective effect of baicalin against OVA+LPS-induced asthma and regulatory effect of baicalin on immunological imbalance of Th17/Treg responses in mice with allergic asthma. This study might provide new insights for understanding immune modulation by baicalin as well as the potential application of baicalin as a drug for asthma treatment.

2. Materials and methods

2.1. Animals

SPF female BALB/c mice, weighing 18–22 g, were purchased from Changchun Yisi Experimental Animal Co. Ltd., China (certificate No. SCXK(Ji) 2011-0004). All mice were maintained in microisolator cages and received food and water ad libitum. Before experimentation, the

mice were acclimatized for a minimum of one week. All animal experimental procedures were performed in accordance with the guidelines of the Ethical Committee for the Experimental Use of Animals at Yanbian University (Yanji, Jilin, China).

2.2. Reagents

Ovalbumin (OVA, Grade V) and lipopolysaccharide (LPS, *Escherichia coli* 055:B5) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Budesonide (BUD) was purchased from AstraZeneca PLC. (London, England). Mouse IL-6, IL-10, IL-17A and IgE ELISA kits were purchased from Biolegend, Inc. (San Diego, CA, USA). Rabbit anti-signal transducer and activator of transcription 3 (STAT3) polyclonal antibody was purchased from Sabbiotech (MD, USA), rabbit anti-forkhead box P3 (FOXP3) polyclonal antibody was purchased from Proteintech (Chicago, USA), HRP-conjugated goat anti-rabbit IgG and β -actin were purchased from Santa Cruz (Santa Cruz, CA, USA). Bicinchoninic acid (BCA), Diaminobenzidine (DAB) kit and chemiluminescent (ECL) reagent kits were purchased from Beyotime Biotech (Shanghai, China). All other chemicals used in this study were analytical grade.

2.3. Baicalin

Baicalin (Batch number: ZL150306582; Molecular formula: $C_{21}H_{18}O_{11}$; Molecular weight: 446.36) was obtained from Nanjing Zelang Medical Technology Co., Ltd. (Nanjing, Jiangsu, China). It was isolated from the dried roots of *Scutellaria baicalensis* Georgi, which was identified macroscopically and microscopically according to the Pharmacopoeia of China, and the voucher specimen (No. 140721) was deposited at the Herbarium of Yanbian University (Yanji, Jilin, China). The extraction and preparation of baicalin were as previously described (Kim et al., 2007). Briefly, the dried roots of *Scutellaria baicalensis* Georgi were cut into small pieces, immersed, extracted with acetone, and then filtrated. The residues were reflux-extracted with 50% aqueous ethanol, and the extracts were concentrated under reduced pressure. Ethanol was added into the concentrated extracts and produced yellow precipitate, which was further purified by recrystallization with aqueous ethanol to yield baicalin. The purify of baicalin was 98.5% based on HPLC analysis equipped with SinoChrom ODS-BP 5 μ m column (Fig. 2). The mobile phase was consisted of methanol-0.1% phosphoric acid (60:40, v/v) and the flow rate was 0.8 ml/min.

2.4. OVA+LPS-induced asthma model and drug treatment

Mice were randomly divided into six groups (n=12): normal group, model group, baicalin treatment groups (10, 25 and 65 mg/kg, respectively), and positive group. Mice were sensitized and challenged with OVA+LPS as previously described (Zhang et al., 2012). The mice were anesthetized with an inhalation of diethyl ether and sensitized with 100 μ g OVA mixed with 0.1 μ g LPS in 50 μ l phosphate-buffered saline (PBS) or PBS alone for normal group on days 1, 7 and 14. The mice were again challenged by intranasal instillations of 50 μ g OVA or PBS alone for normal group from days 22–36 after sensitization. On days 22–36 after sensitization, the mice in baicalin treatment groups were intragastrically treated with baicalin (at doses of 10, 25 and 65 mg/kg, respectively) in 0.5% Sodium Tvlose once per day, and the mice in positive group was treated with BUD (1 mg/kg) by intranasal instillation. The mice in normal group and model group were intragastrically treated with the equal volume of 0.5% Sodium Tvlose.

2.5. Collection of blood and bronchoalveolar lavage fluid (BALF)

Twenty four hours after the last OVA challenge and mice were anesthetized with an inhalation of diethyl ether. Blood was drawn from retro-orbital plexus, sera were collected by centrifugation (4 $^{\circ}$ C, 420 $\times$ g,

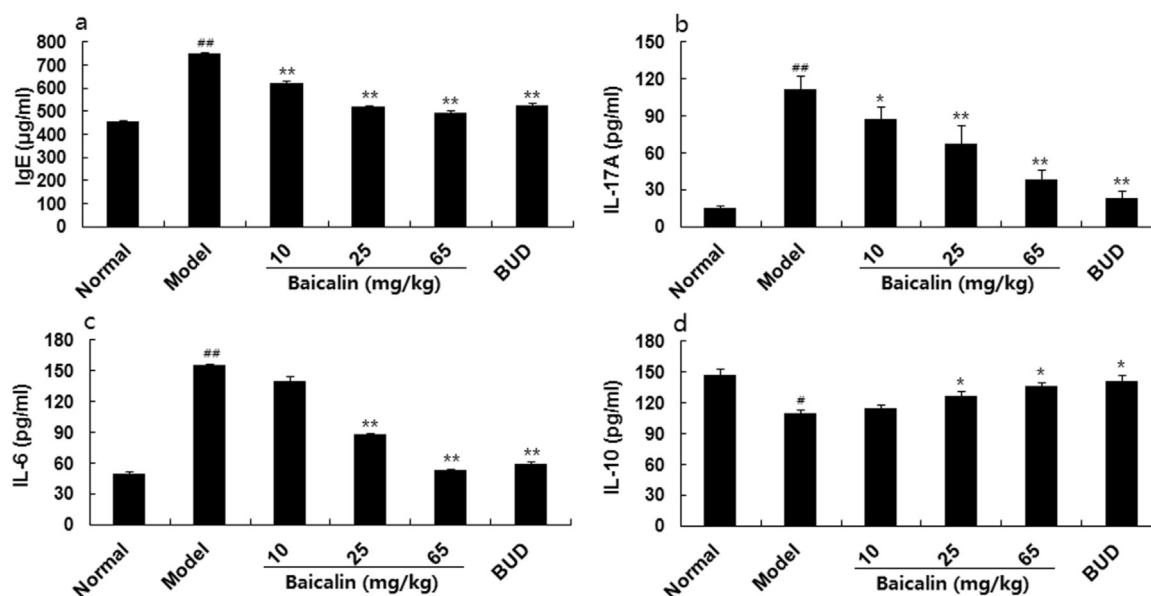


Fig. 4. Effects of baicalin on serum IgE and BALF IL-17A, IL-6 and IL-10 in OVA+LPS-induced asthmatic mice. Mice were sensitized and challenged with OVA combined with LPS, and intragastrically treated with baicalin (at doses of 10, 25 and 65 mg/kg). The levels of serum IgE (a), and BALF IL-17A (b), IL-6 (c) and IL-10 (d) were detected by ELISA kits. The data are expressed as means $\pm$ SEMs. ^{##} $P < 0.01$ vs. normal group; ^{*} $P < 0.05$, ^{**} $P < 0.01$ vs. model group.

15 min) and stored at -80°C for serum determination of IgE. After blood collection, mice were sacrificed by exsanguination. BALF was obtained by intratracheal instillation, and the lungs were lavaged three times with sterile PBS. The collected BALF was centrifuged (4°C , $420\times g$, 15 min), and supernatants were stored at -80°C for subsequent cytokine analysis.

2.6. Determination of organ coefficient of lung tissues

The body weight of mice was measured. The lung tissues were removed and weighed. The organ coefficient of lung tissues were calculated using the following formula: Organ coefficient = wet weight of lung (mg)/body weight (g).

2.7. Detection of serum IgE and BALF cytokine IL-17A, IL-6 and IL-10 levels

The IgE level in sera and cytokine IL-17A, IL-6 and IL-10 levels in BALF were quantified using respective commercially available ELISA kits as per manufacturer's instructions. The concentrations were expressed as $\mu\text{g/ml}$ of sera and $\text{pg}/\mu\text{l}$ of BALF based on the appropriate standard curve.

2.8. Histopathological assessment

The lung and airway tissues of mice were detached and fixed in 10% buffered formalin for 24 h, dehydrated, embedded in paraffin, and then cut into about 3–4 μm thickness sections. The sections were fixed on glass slides, stained with hematoxylin-eosin (H&E) and observed under light microscope for the degree of peribronchial and perivascular inflammation. Periodic acid-Schiff (PAS) staining was performed to estimate the degree of mucus production and goblet cell hyperplasia in the epithelium of the airways.

2.9. Immunohistochemical analysis of STAT3 and FOXP3 expressions in lung tissues

STAT3 and FOXP3 expressions in lung tissues were measured by immunohistochemical analysis. The lung tissues of mice were fixed in 10% buffered formalin, embedded in paraffin, and cut into sections,

and then the sections were processed for immunohistochemical staining with rabbit anti-STAT3 polyclonal antibody and anti-FOXP3 polyclonal antibody followed by avidinbiotin based detection kit and DAB staining, hematoxylin re-staining. STAT3 and FOXP3 expressions were observed by color development under light microscopy.

2.10. Western blot analysis of STAT3 and FOXP3 in lung tissues

The lung tissues of mice were removed and homogenized, and lysed in extraction lysis buffer followed by quick freezing. The protein concentration was determined using BCA protein assay reagent according to the manufacturer's protocols. Equal amounts of protein were separated on 10% SDS-PAGE, and transferred to PVDF membrane. The immunoblots were blocked with Tween 20/Tris-buffered saline (TTBS) with 5% skim milk, and followed by incubation with rabbit anti-STAT3 (1:1000) and anti-FOXP3 (1:1000) polyclonal antibodies overnight at 4°C . Immunoblots were washed with TTBS and incubated with secondary antibodies (goat anti-rabbit IgG-HRP, 1:1000) for 1 h at room temperature. Immunoblots were washed with TTBS again and then developed by ECL. Images were scanned with a Densitometer Scanner (GS800, Bio-Rad) and analyzed using Quantity One software (Bio-Rad). To normalize for protein loading, antibodies directed against β -actin were used, and STAT3 and FOXP3 protein levels were expressed as a relative value to that of β -actin.

2.11. Statistical analysis

All data are expressed as means $\pm$ SEMs. Statistical analysis was performed using one-way ANOVA followed by a multiple comparison test. Statistical significance was accepted at $P < 0.05$.

3. Results

3.1. Effects of baicalin on organ coefficient of lung tissues

As shown in Fig. 3, OVA mixed with LPS obviously increased the organ coefficient of lung tissues compared with that of normal group ($P < 0.01$). However, baicalin at 25, 65 mg/kg and BUD obviously reduced the organ coefficient of lung tissues in OVA+LPS-sensitized and challenged mice ($P < 0.05$ or $P < 0.01$).

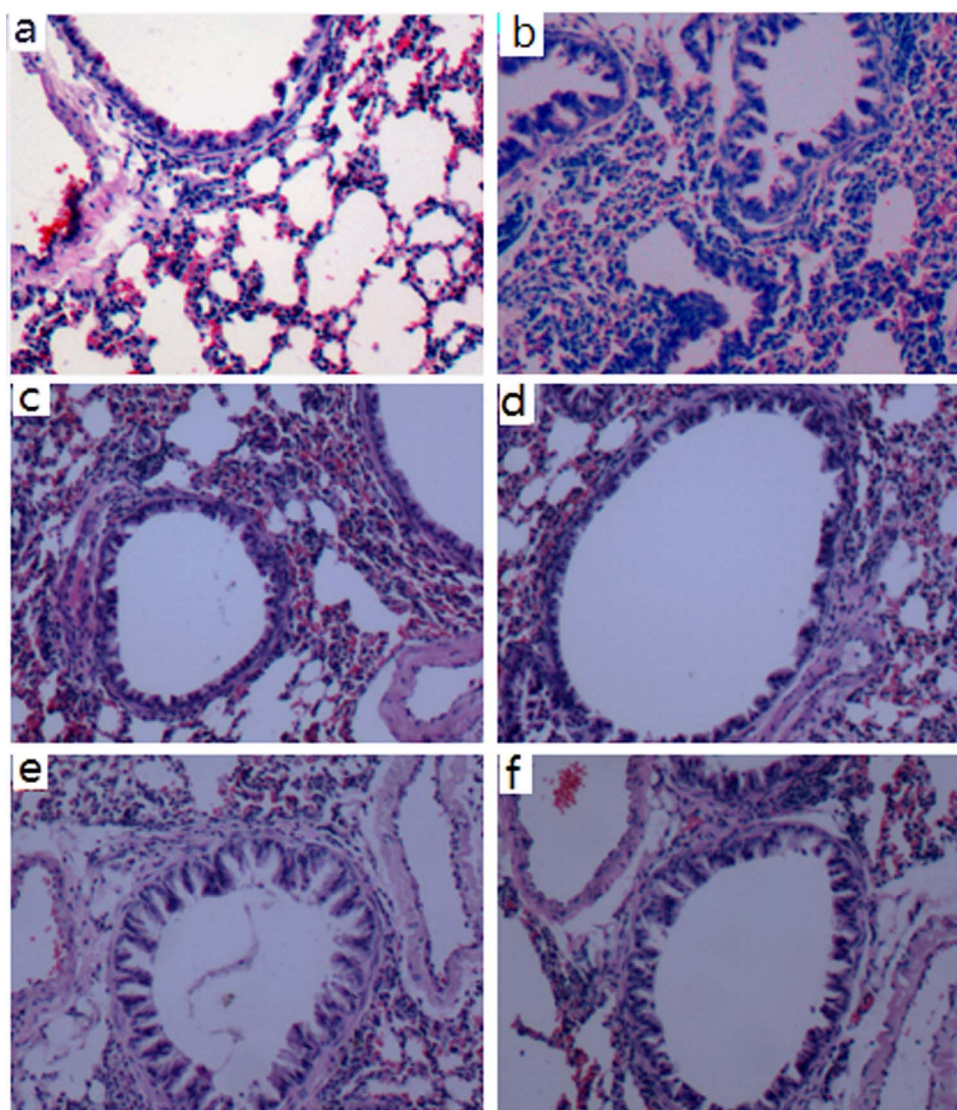


Fig. 5. Effects of baicalin on histopathological changes of lung tissues in OVA+LPS-induced asthmatic mice ($\times 200$, H & E staining). Mice were sensitized and challenged with OVA combined with LPS, and intragastrically treated with baicalin (at doses of 10, 25 and 65 mg/kg). The inflammatory responses of lung tissues were observed under light microscopy by H & E staining. (a) Normal group; (b) Model group; (c) Baicalin 10 mg/kg group; (d) Baicalin 25 mg/kg group; (e) Baicalin 65 mg/kg group; (f) BUD group.

3.2. Effects of baicalin on IgE level in sera and cytokine IL-17A, IL-6 and IL-10 levels in BALF

As shown in Fig. 4, OVA mixed with LPS obviously promoted IgE production in sera, IL-17A and IL-6 production in BALF ($P < 0.01$), whereas inhibited IL-10 production in BALF compared with that of normal group ($P < 0.05$). However, baicalin at 10, 25 and 65 mg/kg obviously suppressed OVA+LPS-sensitized and challenged serum IgE level (Fig. 4a, $P < 0.01$), baicalin at 10, 25 and 65 mg/kg obviously reduced OVA+LPS-induced BALF IL-17A production (Fig. 4b, $P < 0.05$ or $P < 0.01$), baicalin at 25 and 65 mg/kg obviously reduced OVA+LPS-induced BALF IL-6 production (Fig. 4c, $P < 0.01$), and baicalin at 25 and 65 mg/kg obviously increased the decrease of BALF IL-10 production induced by OVA+LPS (Fig. 4d, $P < 0.05$). BUD also obviously inhibited IgE, IL-17A, IL-6 and promoted IL-10 production ($P < 0.05$ or $P < 0.01$).

3.3. Effects of baicalin on histopathological changes of lung and airway tissues

As shown in Figs. 5a and 6a, no histopathological changes of lung and airway tissues were found in normal group. The significant

inflammatory changes characterized by a large number of inflammatory cell infiltration into the peribronchial and perivascular areas (H & E staining), mucus overproduction and goblet cell hyperplasia in the bronchial airways (PAS staining) were observed in OVA+LPS-sensitized and challenged lung and airway tissues of mice (Figs. 5b and 6b). In contrast, baicalin and BUD obviously attenuated the degree of inflammatory responses in the peribronchial and perivascular areas (Fig. 5c-f), mucus overproduction and goblet cell hyperplasia in the airways (Fig. 6c-f).

3.4. Effects of baicalin on STAT3 and FOXP3 expressions in lung tissues

We further examined STAT3 and FOXP3 expressions in lung tissues by immunohistochemistry and western blot methods. Immunohistochemistry study (Fig. 7) showed that STAT3 expression was obviously upregulated, whereas FOXP3 expression was obviously down-regulated in OVA+LPS-sensitized and challenged lung tissues of mice compared with that of normal group (brown yellow granules indicate positive reaction). Treatment with baicalin and BUD inhibited OVA+LPS-induced STAT3 expression and promoted FOXP3 expression. Western blot study (Fig. 8) also showed that baicalin and BUD

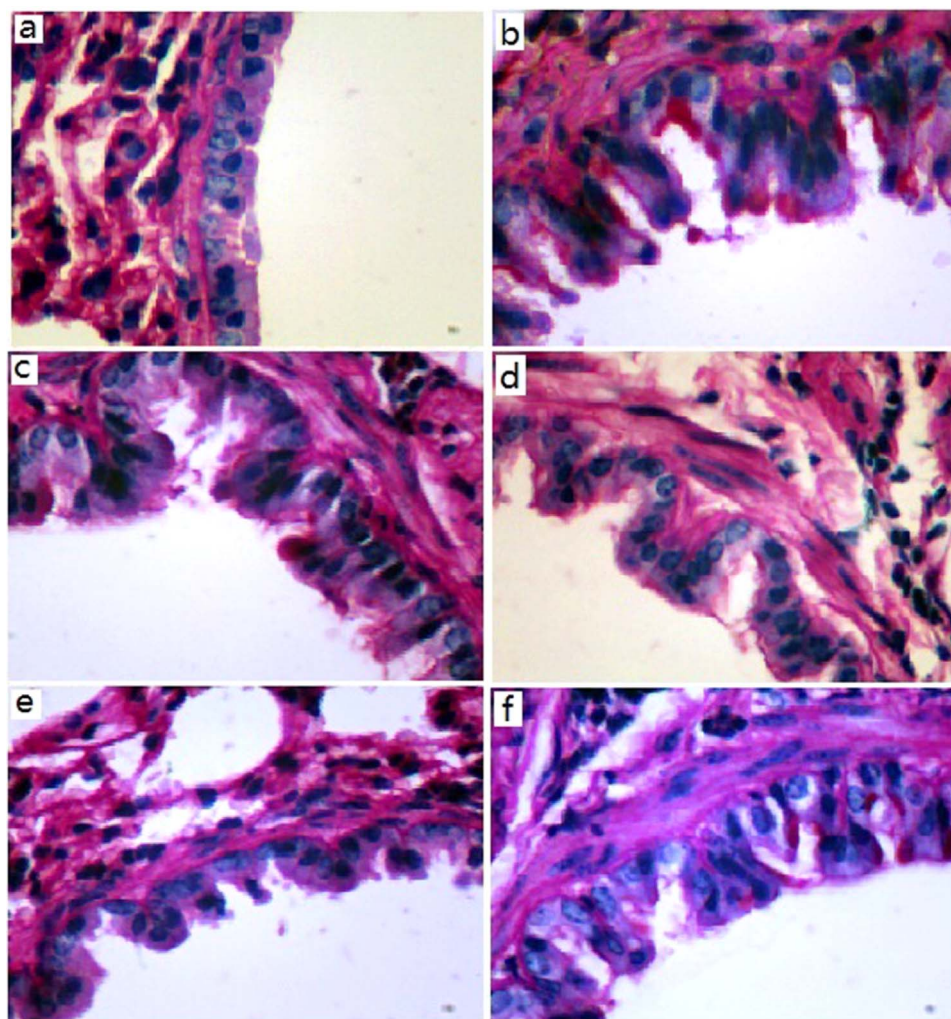


Fig. 6. Effects of baicalin on histopathological changes of airway tissues in OVA+LPS-induced asthmatic mice ($\times 400$, PAS staining). The mucus production and goblet cell hyperplasia in airway tissues were observed under light microscopy by PAS staining. (a) Normal group; (b) Model group; (c) Baicalin 10 mg/kg group; (d) Baicalin 25 mg/kg group; (e) Baicalin 65 mg/kg group; (f) BUD group.

obviously inhibited OVA+LPS-induced STAT3 expression and promoted FOXP3 expression, it is consistent with the result of immunohistochemistry.

4. Discussion

Allergic asthma is rather complex and heterogeneous in pathogenesis involving a variety of immune cells and cytokines (Holgate, 2012). Th2 cells produce cytokines such as IL-4, IL-5, and IL-13, which contribute to eosinophilia and Ag-specific IgE production, whereas Th1 cells suppress Th2 immune responses by secreting IFN- γ (Wegmann, 2009; Nakajima and Hirose, 2010; Ma et al., 2014). Thus, it has always been thought that Th1/Th2 imbalance is implicated in the pathogenesis of asthma and plays a crucial role. Th17 cells were recently discovered as another T lymphocyte subset differing from Th1 and Th2 cells (Kimura and Kishimoto, 2011). The discovery of Th17 cells further perfects the pathogenesis of asthma. In the inflammatory process of allergic asthma, Th17 cells produce cytokines IL-17 and IL-6, and decrease transcription factor Foxp3 which is expressed by Treg cells, causing the Th17/Treg imbalance (Wan and Wei, 2015; Lim et al., 2016). Therefore, in addition to Th1/Th2 imbalance, Th17/Treg imbalance also plays an important role in the pathogenesis of asthma and is becoming a focus for the treatment and prevention of asthma (Hua et al., 2014). In the current study, OVA+LPS intranasal sensitization model of mice based on Th17/Treg imbalance was established to

investigate the potential protective effect of baicalin against asthma and involved immunoregulatory mechanism. Baicalin effectively protects against OVA+LPS-induced allergic asthma in mice by regulating the immunological imbalance of Th17/Treg responses.

Th17 cells play an important role in the pathogenesis of asthma by producing cytokines such as IL-17A, IL-6 and etc. IL-17A is the main effector secreted by Th17 cells, and acts as a potent mediator of immune reactions by increasing chemokine production in various tissues, facilitating recruitment of inflammatory cells to inflammation areas and promoting the inflammatory response (Monteleone et al., 2012). It has been reported that IL-17 mRNA and protein levels in the lung tissues, serum, BALF, and sputum are significantly higher in asthma patients (Bullens et al., 2005; Sun et al., 2005). Cytokine IL-16 also plays an active role in the pathogenesis of asthma. IL-6 mediates Th17 cell differentiation through STAT3 activation, it interacts with TGF- β , and promotes Th17 cell differentiation and inhibits Treg cells proliferation (Rincon and Irvin, 2012). Increased levels of IL-6 in sera and BALF have been found in asthmatic mouse models and asthmatic patients (Rincon and Irvin, 2012). Our present study showed BALF IL-17A and IL-6 levels were significantly increased in OVA+LPS-induced allergic mice, and treatment with baicalin significantly inhibited cytokine IL-17 and IL-6 secretion. Whereas IL-10 is recognized as a potential anti-inflammatory cytokine, which can inhibit the synthesis and release of pro-inflammatory cytokines and inhibit the synthesis of inflammatory cells (Tang et al., 2012). IL-10 deficiency induces

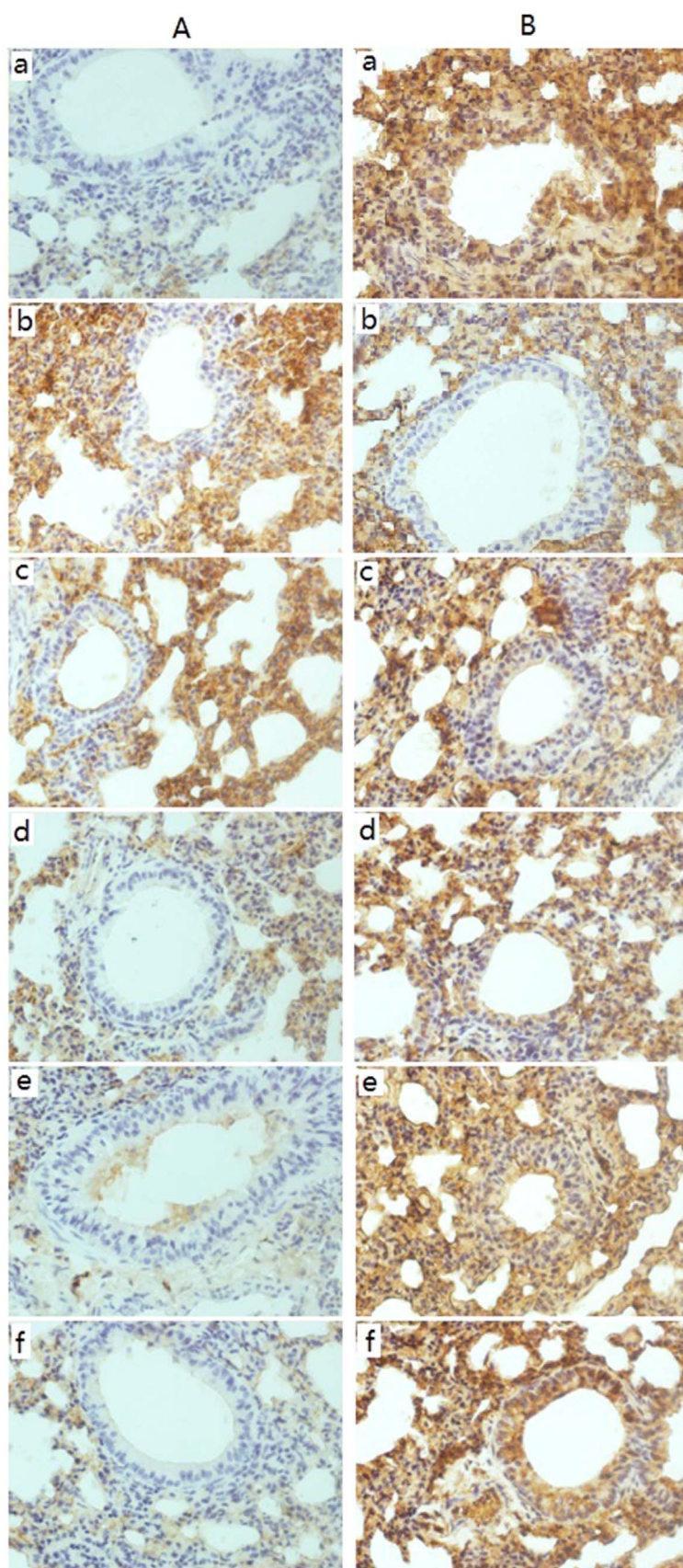


Fig. 7. Effects of baicalin on STAT3 and FOXP3 expressions in lung tissues in OVA+LPS-induced asthmatic mice ($\times 200$, immunohistochemical analysis). Mice were sensitized and challenged with OVA combined with LPS, and intragastrically treated with baicalin (at doses of 10, 25 and 65 mg/kg). STAT3 (A) and FOXP3 (B) expressions in lung tissues were measured by immunohistochemical analysis. (a) Normal group; (b) Model group; (c) Baicalin 10 mg/kg group; (d) Baicalin 25 mg/kg group; (e) Baicalin 65 mg/kg group; (f) BUD group.

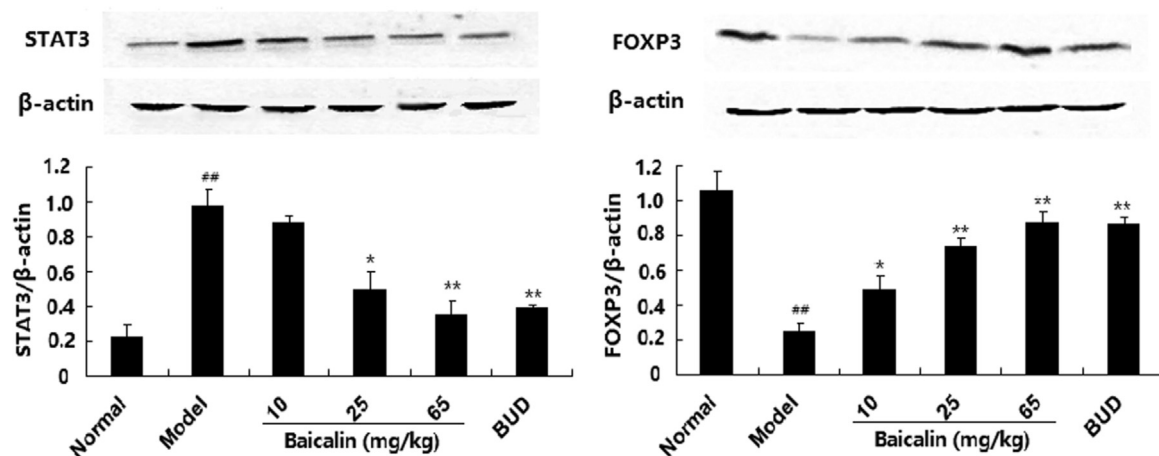


Fig. 8. Effects of baicalin on STAT3 and FOXP3 expressions in lung tissues in OVA+LPS-induced asthmatic mice (western blot analysis). Mice were sensitized and challenged with OVA combined with LPS, and intragastrically treated with baicalin (at doses of 10, 25 and 65 mg/kg). STAT3 and FOXP3 expressions in lung tissues were measured by western blot. STAT3 and FOXP3 protein expressions were normalized to β -actin using a densitometer. The data are representative of three independent experiments and expressed as mean $\pm$ SEMs. ^{##} P < 0.01 vs. normal group; ^{*} P < 0.05, ^{**} P < 0.01 vs. model group.

differentiation to form Th17 cells and reduces Treg cell formation, causing infection, allergy, and autoimmunity (Chaudhry et al., 2011). The present study showed that treatment with baicalin increased the decrease of BALF IL-10 production induced by OVA+LPS.

In addition, the inhibition of baicalin on inflammatory cytokines attributes to the initial suppression of an allergen-specific IgE response. In many allergic inflammatory diseases, elevations in the plasma level of IgE induce the degranulation of mast cells via the cross-linking of allergen-specific IgE, and it has been viewed as a target for novel immunological drug development in allergic asthma (Lee et al., 2011). There is a close and highly significant relationship between serum IgE and the pathogenesis of asthma (Holgate et al., 2005). IgE can stimulate the inflammatory cells to secrete leukotrienes, histamine and other inflammatory mediators, causing mucus secretion, inflammatory cell infiltration and other symptoms of asthma (Bax et al., 2012). In this study, mice exposed to OVA+LPS exhibited a significant increase of serum IgE, however, treatment with baicalin significantly decreased serum IgE concentration in allergic asthma of mice. It suggests that baicalin has certain effect on OVA+LPS-induced allergic asthma that developed in an IgE-dependent manner. Furthermore, in the development of allergic asthma, the prominent histopathological changes of lung and airway inflammation are characterized by influx of a large number of inflammatory cells, mucus hypersecretion, and goblet cell hyperplasia as well as by the thicknesses of alveolar septum. In this study, these prominent histopathological features were found in OVA+LPS-induced allergic asthma of mice. In contrast, treatment with baicalin successfully abated lung inflammation, reduced mucus hypersecretion and goblet cell hyperplasia in the airways.

STAT3, a transcription factor essential for Th17 cell differentiation, is required for Treg cell-mediated control of Th17 cell responses (Chaudhry et al., 2011). STAT3 can be activated by inflammatory cytokines such as IL-6 and IL-23 which serve as critical inducers of Th17 cell responses, and enable Treg cells-mediated suppression of Th17 cell-dependent inflammation (McGeachy et al., 2007). Thus, STAT3 is critical for cytokine signaling and acts as a novel epithelial regulator in allergic response, targeting this molecule may provide the basis for a novel asthma therapy (Simeone-Penney et al., 2007). Treg cells play important roles in maintaining immunological self-tolerance and anti-inflammation (Pereira et al., 2014). Furthermore, they also have a mutual inhibitory relationship with Th17 cells in terms of differentiation, development and function (Hua et al., 2014). The transcription factor FOXP3, which is specifically expressed by Treg cells, has been considered as a lineage-specification factor or master

regulator for Treg cells and crucially regulates the development and function of Treg cells. It is reported that FOXP3 deletion or mutations in humans and mice cause severe autoimmune and inflammatory diseases, as a result of the failure in generating Treg cells. The present immunohistochemistry and western blot results demonstrated that treatment with baicalin markedly suppressed OVA+LPS-induced STAT3 expression and promoted FOXP3 expression in the lung tissues of mice.

In summary, our study demonstrates that baicalin decreases organ coefficient of lung, inhibits serum IgE and BALF IL-7A and IL-6 secretion, promotes BALF IL-10 secretion, alleviates inflammatory responses and mucus secretion of lung tissues and airways in OVA+LPS-induced allergic asthma of mice. Furthermore, baicalin suppresses STAT3 expression and promotes FOXP3 expression in lung tissues of mice. It suggests that baicalin may restore the balance of Th17/Treg responses by suppressing IL-17, IL-6, IgE secretions and STAT3 expression, and promoting IL-10 secretion and FOXP3 expression, thus has a protective effect against OVA+LPS-induced allergic asthma of mice.

Author contributions

X.M. Zhang designed the experiments; L. Xu, J.X. Li, Y. Zhang and P. Zhao performed the experiments and analyzed the data; X.M. Zhang and L. Xu wrote the manuscript.

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