ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
October 2023; 22(5):430-439.
DOI: 0.18502/ijaai.v22i5.13993

Amygdalin Improves Allergic Asthma via the Thymic Stromal Lymphopoietin–dendritic Cell–OX40 Ligand Axis in a Mouse Model

Wen Cui, Huan Zhou, Ya-zun Liu, Yan Yang, Yi-zhong Hu, Zhao-peng Han, Jian-er Yu, and Zheng Xue

Department of Pediatrics, Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai, China

Received: 23 February 2023; Received in revised form: 26 September 2023; Accepted: 26 September 2023

ABSTRACT

Asthma, characterized by persistent inflammation and increased sensitivity of the airway, is the most common chronic condition among children. Novel, safe, and reliable treatment strategies are the focus of current research on pediatric asthma. Amygdalin, mainly present in bitter almonds, has anti-inflammatory and immunoregulatory potential, but its effect on asthma remains uninvestigated. Here, the impact of amygdalin on the thymic stromal lymphopoietin (TSLP)–dendritic cell (DC)–OX40L axis was investigated.

A BALB/c mouse model for allergic asthma was established using the ovalbumin-sensitization method. Amygdalin treatment was administered between days 21 and 27 of the protocol. Cell numbers and hematoxylin and eosin (H&E) staining in bronchoalveolar lavage fluid (BALF) were used to observe the impact of amygdalin on airway inflammation. TSLP, IL-4, IL-5, IL-13, and IFN-γ concentrations were determined via Enzyme-linked immunosorbent assay (ELISA). TSLP, GATA-3, and T-bet proteins were measured using western blotting. Cell-surface receptor expression on DCs (MHC II, CD80, and CD86) was assessed via flow cytometry. OX40L mRNA and protein levels were detected using western blotting and qRT-PCR, respectively.

Amygdalin treatment attenuated airway inflammation decreased BALF TSLP levels, inhibited DC maturation, restrained TSLP-induced DC surface marker expression (MHCII, CD80, and CD86), and further decreased OX40L levels in activated DCs. This occurred together with decreased Th2 cytokine levels (IL-4, IL-5, and IL-13) and GATA3 expression, whereas Th1 cytokine (IFN-γ) levels and T-bet expression increased.

Amygdalin thus regulates the Th1/Th2 balance through the TSLP–DC–OX40L axis to participate in inflammation development in the airways, providing a basis for potential allergic asthma treatments.

Keywords: Airway inflammation; Amygdalin; Asthma; Dendritic cells; OX40 Ligand; Th1-Th2 balance; Thymic stromal lymphopoietin

Corresponding Author: Zheng Xue, MD;
Department of Pediatrics, Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai, China. Tel: (+86 181) 1607 0951, Fax: (+86 21) 5668 9828, E-mail: xuezheng@shutcm.edu.cn

*The first and second authors contributed equally to this study

INTRODUCTION

Asthma is a chronic condition marked by persistent inflammation and increased airway reactivity, resulting in symptoms that include cough, wheezing, breathlessness, and chest tightness.[1] It is the most frequently occurring chronic ailment in children, affecting approximately 14% of children worldwide, and its prevalence is increasing. Among chronic respiratory diseases, asthma ranks second as a cause of mortality.[1] Since the 1970s, inhaled corticosteroids (ICSs) have been widely recognized as the primary treatment for asthma.[2] However, drug-related side effects include ophthalmic effects, hypothalamic–pituitary–adrenal axis suppression, diabetes, respiratory infections, slower growth, and osteoporosis.[3] The likelihood of experiencing these negative consequences is particularly significant in cases of severe asthma, impacting approximately 5% to 10% of patients who necessitate high dosages of ICSs.[4] Therefore, investigations of novel, safe, and reliable treatment strategies are the focus of current research on pediatric asthma.

Allergic asthma typically begins during childhood, and it is linked to immune responses mediated by T helper 2 (Th2) cells.[5] Thymic stromal lymphopoietin (TSLP), a cytokine predominantly released by cells of bronchial and lung epithelium, plays a central role in preserving immune balance and modulating type 2 inflammatory reactions in different allergy conditions.[6] Targeting TSLP and its signaling pathway presents a promising therapeutic approach.[7,8] Dendritic cells (DCs) are widely regarded as pivotal regulators of immune response initiation in asthma.[9] TSLP plays a crucial role as an upstream cytokine for DCs,[10] promoting expression of the OX40 ligand (OX40L) and subsequently inducing differentiation of Th2 cells.[11] Thus, the TSLP–DC–OX40L pathway appears to be involved in airway inflammation in asthma.[12]

Natural traditional herbal medicines and plant-derived metabolites are well known for treating asthma, with low toxicity.[13-15] Amygdalin, a prominent bioactive compound found in Chinese raw bitter almonds, has long been widely used in various diseases.[16] Figure 1A depicts a structural representation of amygdalin, and its molecular formula is $C_{20}H_{27}NO_{11}$ (MW: 457.43). Amygdalin has antitumor, antifibrosis, pain-relieving, immunomodulatory, and anti-atherosclerosis activities.[17] Notably, amygdalin has the ability to reduce epithelial-mesenchymal transition, inflammation, and apoptosis of bronchial airway epithelial cells.[18] Nevertheless, the specific anti-allergic asthma effects of amygdalin remain unexplored. Here, we sought to explore the impact of amygdalin on asthma, as well as its possible molecular mechanisms, using an ovalbumin (OVA)-induced asthma mouse model.

MATERIALS AND METHODS

Animals, Asthma Mouse Model Establishment, and Treatment Protocol

Male BALB/c mice (6 to 8 weeks), purchased from Shanghai Slac Laboratory Animal Co., Ltd, had unrestricted access to food and water in standard transparent plastic cages. Housing conditions were maintained at 21℃ with a 12-hour light-dark cycle. All animal experiments were approved by the Institutional Animal Care and Use Committee of Shanghai Municipal Hospital of Traditional Chinese Medicine (No. 2022017).

Mice were randomly assigned to 4 groups, each containing 6 mice, as follows: control (normal saline; NS), OVA (OVA+ NS), DEX (OVA + dexamethasone; Dex), and amygdalin (OVA+amygdalin). We established an OVA-induced asthma mouse model, conducted as described previously[15] with modifications (Figure 1B). In the OVA, DEX, and amygdalin groups, mice were sensitized via intraperitoneal injection of OVA (50 μg, Sigma-Aldrich, St. Louis, USA) emulsified in aluminum peroxide (1 mg, Sigma-Aldrich) on day 0 and 14. The challenge was performed with 5% OVA inhalation for 30 minutes using an ultrasonic nebulizer (OMRON Co., Tokyo, Japan) over days 21 to 27. Mice in the DEX or amygdalin group were administered 200 μL Dex (2 mg/kg, Shanghai Xinyi Pharmaceutical Co., Ltd., Shanghai, China) or amygdalin (15 mg/kg, Sigma-Aldrich) via gavage, 1 hour before the challenge. The OVA group was sham-treated with NS. The control group underwent sensitization, challenge, and treatment, all with NS. All mice were euthanized 24 hours following the last OVA challenge on day 28.

Bronchoalveolar Lavage Fluid (BALF) Collection and Total Cell Counting

BALF was obtained 24 hours after the final challenge, as described previously.[19] Briefly, mice were

euthanized, and the tracheae were isolated. Phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin was instilled into the lungs 3 times, BALF was collected and centrifuged at 1250*g* for 5 minutes at 4°C. The supernatants were gathered and preserved at −80 ℃ for analysis. To perform differential cell counts, the cell pellets were resuspended in 200 μL NS and centrifuged onto slides using a cytospin device (Shandon Cytospin 4, Thermo Fisher Scientific, USA) at 200*g* for 10 minutes at 4°C. The slides were then stained with Wright-Giemsa Staining Solution (Beyotime, Shanghai, China) to determine total cell counts.

Enzyme-linked Immunosorbent Assay (ELISA)

The concentration of TSLP, interferon (IFN)-γ, interleukin (IL)-4, IL-5, and IL-13 in the BALF supernatant were quantified using ELISA kits (R&D Systems, Minneapolis, USA) following the manufacturer's instructions.

Lung Histopathology

Lung tissue was fixed with 10% formaldehyde, dehydrated, made transparent, dipped in wax, embedded, sliced into paraffin slices with a 5 mm thickness, and stained with hematoxylin and eosin (H&E; BASO, Shanghai, China). Finally, lung tissue samples were examined under an optical microscope (Olympus 2H12003, Tokyo, Japan) to assess pathological changes.

Western Blotting

Briefly, lung tissues were fragmented into small pieces and homogenized in radio-immunoprecipitation assay buffer (RIPA) lysis buffer (Beyotime). The lyzed samples were centrifuged at 12000*g* for 15 minutes at 4℃, and the supernatant was collected. Protein concentrations were assessed utilizing a bicinchoninic acid (BCA) protein assay kit (Beyotime). Protein lysates (25 μg) were loaded onto an 8–15% sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel and electrophoresed onto polyvinylidene fluoride (PVDF) membranes (Millipore, Germany). Then, the membranes were blocked with 5% skim milk for 1 hour at 23℃.

The blots were incubated at 4°C overnight with antibodies against TSLP, OX40L, GATA-3, T-bet, and GAPDH (Abcam, USA). After washing with 1×Tris-Buffered Saline with Tween (TBST), the membranes were exposed to goat anti-rabbit IgG or goat anti-mouse IgG (Abcam). Finally, an enhanced chemiluminescence reagent kit (Beyotime) was used for color development. Relative protein quantification was performed using Quantity One software (Bio-Rad, USA). All experiments were independently repeated thrice.

Generation of DCs Derived from Bone Marrow

On day 28, the mice were euthanized, and the femur and tibia were removed. Bone marrow cells were flushed out using a syringe containing 5 mL of PBS. Red blood cell elimination was carried out using Red Blood Cell Lysis Buffer (Solarbio, China). The remaining cells were resuspended in Roswell Park Memorial Institute 1640 (RPMI1640) medium (Hyclone, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA), 1 mM sodium pyruvate (Solarbio), 1% Non-essential amino acids (NEAA) (Gibco), 50 μM 2-mercaptoethanol (Gibco), 1% penicillin-streptomycin (Solarbio), recombinant murine IL-4 (1000 U/mL; Sigma) and granulocyte-macrophage colony-stimulating factor (GM-CSF) (500 U/mL; Sigma) with a concentration of 2×10^5/mL. Cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2. Every 2 days, half of the medium was exchanged with fresh, complete medium. Cell morphology was observed on days 0, 3, 6, and 9. Cells were collected and resuspended in RPMI1640 on day 9.

Flow Cytometric Analysis of DC Surface Markers

DCs were centrifuged, washed, and resuspended in PBS. Single-cell suspensions of DCs were stained with Anti-Mouse major histocompatibility complex class II (MHCII)-FITC (eBioscience, USA), Anti-mouse cluster of differentiation (CD80)-FITC (BD Biosciences, USA), and Anti-mouse CD86-FITC (BD Biosciences) in the dark at 4°C for 1 hour. A total of 10,000 cells per sample were measured using a flow cytometer (BD Accuri™ C6, BD Biosciences).

Quantitative Real-time Polymerase Chain Reaction (PCR)

Total RNA from DCs was extracted using TRIzol reagent (Invitrogen, California, USA) following the manufacturer's instructions. Subsequently, reverse transcription was carried out using Superscript III Reverse Transcriptase (Fermentas, CAN). Real-time analysis was performed with Maxima SYBR Green/ROX qPCR Mix (Thermo Fisher, USA) with a PCR detection system (Applied Biosystems™ 7500,

Applied Biosystems, USA). PCR was performed using the following specific primers: OX40L (forward, 5′-ACCCTCCAATCCAAAGAC-3′; reverse, 5′-TCGCACTTGATGACAACC-3′) and GAPDH (forward, 5′-ATCACTGCCACCCAGAAG-3′; reverse, 5′- TCCACGACGGACACATTG-3′). *GAPDH* was used for normalization, and relative gene expression was estimated using the $2^{-\Delta\Delta Ct}$ method.

Statistical Analysis

All data are presented as the mean ± standard deviation (SD). Statistical comparisons were performed via one-way analysis of variance (ANOVA) using SPSS software (version 18.0, USA), followed by Dunnett's multiple comparison test. Results with $p<0.05$ were considered statistically significant.

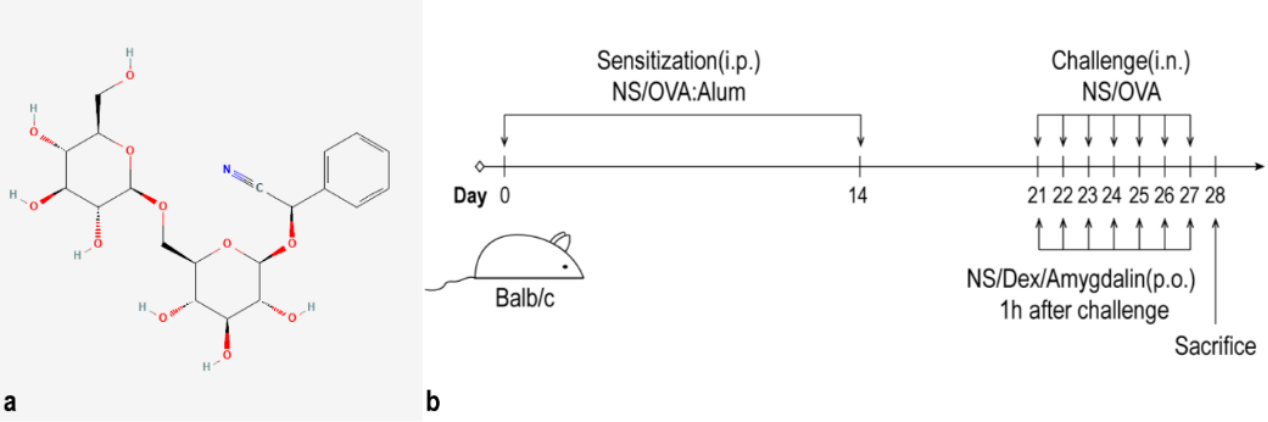

Figure 1. Chemical structure of amygdalin and experimental design of the murine asthma model and treatment with amygdalin. (A) Illustration of the amygdalin molecule, as obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov). (B) Mice were sensitized on days 0 and 14 through intraperitoneal injection of ovalbumin (OVA; 50 µg/L mg aluminum peroxide) and challenged on days 21 to 27 via OVA nebulization (5% OVA; 30 min). Amygdalin (15 mg/kg) or dexamethasone (2 mg/kg) treatments occurred from day 21 to 27 of the protocol. Mice were euthanized on day 28.

RESULTS

Amygdalin Attenuates Airway Inflammation in OVA-induced Allergic Asthma

To evaluate the impact of amygdalin on airway inflammation, inflammatory cell counts in BALF were quantified (Figure 2A). Compared with the control group, total counts of cells, as well as macrophages, eosinophils, neutrophils, and lymphocytes, exhibited a significant increase in OVA-sensitized mice ($p<0.01$). However, Dex or amygdalin administration led to a notable decrease in both total and inflammatory cells ($p<0.01$ and $p<0.05$, respectively).

Furthermore, H&E staining of lung tissues was performed (Figure 2B). Lung tissue histology of OVA-treated mice exhibited notable indicators of increased mucus hypersecretion, inflammatory cell invasion, and goblet cell proliferation. However, histopathological changes were significantly ameliorated in DEX or Amygdalin groups. Collectively, these findings suggest that amygdalin might alleviate airway inflammation in OVA-challenged mice.

Amygdalin Downregulates TSLP Levels in OVA-Induced Allergic Asthma

Western blotting revealed significant elevation in TSLP protein levels in lung tissues of the OVA group ($p<0.01$, Figures 3A and 3B). However, TSLP levels were markedly reduced in both DEX and amygdalin groups ($p<0.01$, Figures 3A and 3B).

Meanwhile, TSLP levels in BALF showed consistent results (Figure 3C). ELISA showed that TSLP cytokines were markedly enhanced in the OVA group ($p<0.01$). The administration of Dex or amygdalin led to a notable decrease in TSLP levels ($p<0.01$). These data indicate that amygdalin could reduce TSLP expression within the respiratory passages of mice experiencing allergic asthma.

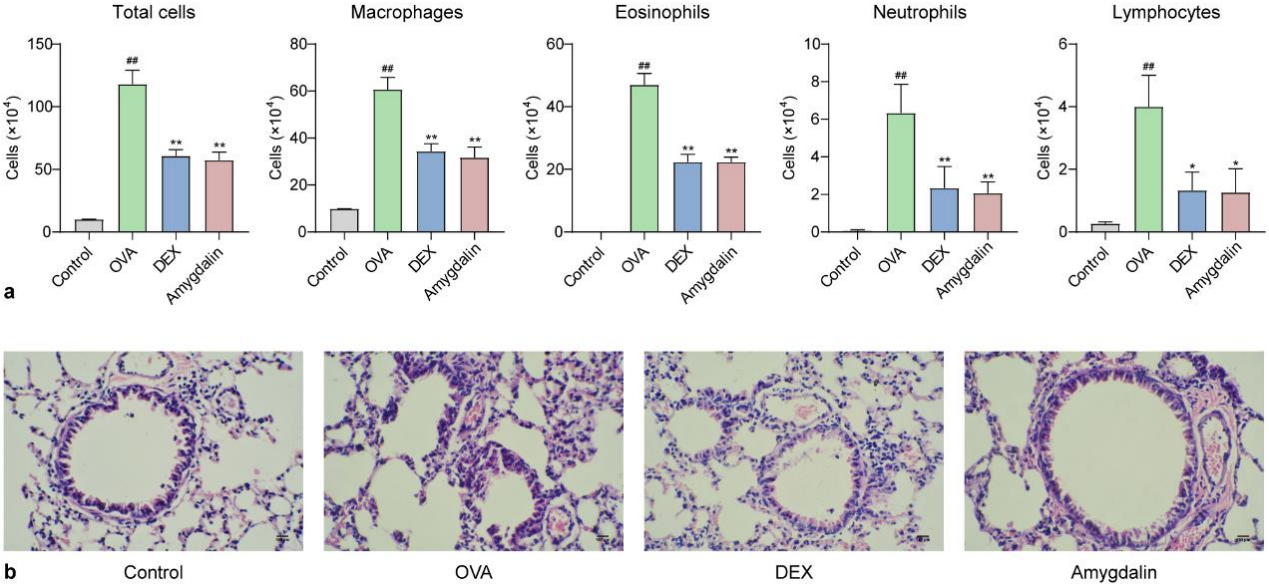

Figure 2. Impact of amygdalin on airway inflammation. (A) Effect of amygdalin on cell counts in bronchoalveolar lavage fluid. # or * p<0.05; ## or ** p<0.01; # vs. Control group; * vs. ovalbumin (OVA) group. (B) Histopathological changes in the lung tissues stained with hematoxylin and eosin. Bars = 100 µm.

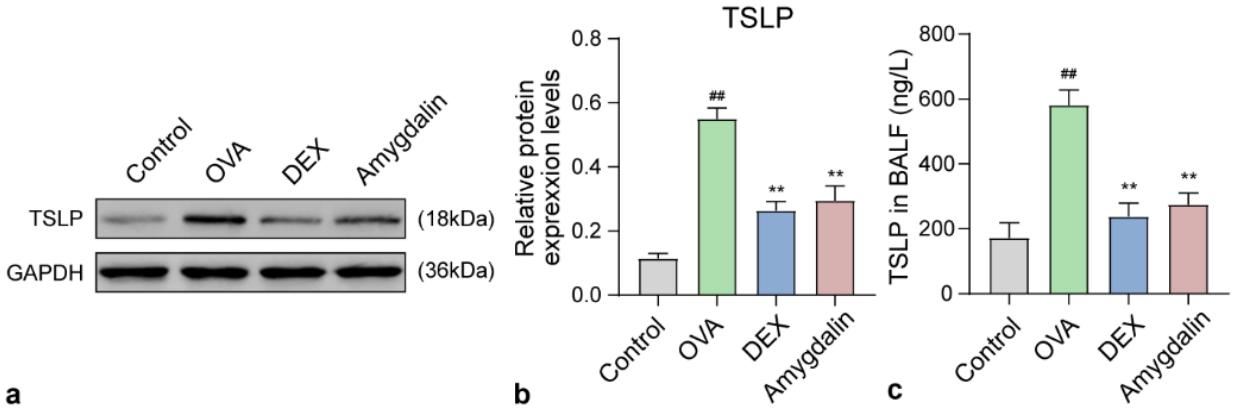

Figure 3. Amygdalin counteracts thymic stromal lymphopoietin (TSLP) expression in ovalbumin (OVA)-sensitized mice. (A and B) TSLP expression in lung tissues was assessed using western blotting, with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) utilized as the internal control. (C) The concentration of TSLP in bronchoalveolar lavage fluid (BALF) was measured using enzyme-linked immunosorbent assay (ELISA). ## or ** p<0.01; # vs. Control group; * vs. OVA group.

Amygdalin Slows Dendritic Cell Maturation and Inhibits the DC–OX40L Axis

We assessed the cell-surface receptors of DCs (MHCII, CD80, and CD86), which are required for T-cell activation (Figure 4). Results showed that MHCII, CD80, and CD86 (Figures 4A to 4C) expressed by DCs were significantly increased in the OVA group (p<0.01). Dex or amygdalin administration resulted in a reduction in MHCII, CD80, and CD86 expression, indicating a notable impact on the maturation and activation of DCs mediated by amygdalin.

We further evaluated the capacity of amygdalin to activate DCs to produce OX40L (Figure 5). Western blotting showed that OX40L protein levels in DCs were

elevated in the OVA group (p<0.01, Figures 5A and 5B). Treatment with Dex or amygdalin resulted in significantly decreased OX40L levels (p<0.01). OX40L mRNA levels in DCs showed the same results (Figure 5C). These findings demonstrate that amygdalin inhibited DC maturation, restrained the TSLP-induced DC surface marker expression, and further decreased OX40L levels in activated DCs.

Th2 cytokine (IL-4, IL-5, and IL-13) levels in BALF exhibited a significant increase in the OVA group (p<0.01, Figure 6A). Conversely, levels of Th1 cytokine (IFN-γ) were markedly decreased (p<0.01, Figure 6B). Treatment with Dex or amygdalin restored Th2 cytokine and Th1 cytokine expression (p<0.01). These results indicate that amygdalin could attenuate the Th2 response in the airways of mice with OVA-induced allergic asthma.

Moreover, in the OVA group, an increase in the protein levels of GATA-3, a critical regulator of Th2 cells, was observed, while a decrease in the protein levels of T-bet, a crucial regulator of Th1 cells, was noted (p<0.01, Figure 6C and 6D). GATA-3 expression was decreased, and T-bet expression was increased after treatment with Dex or amygdalin (p<0.01). These findings align with the impact of amygdalin on the production of Th1 and Th2 cytokines.

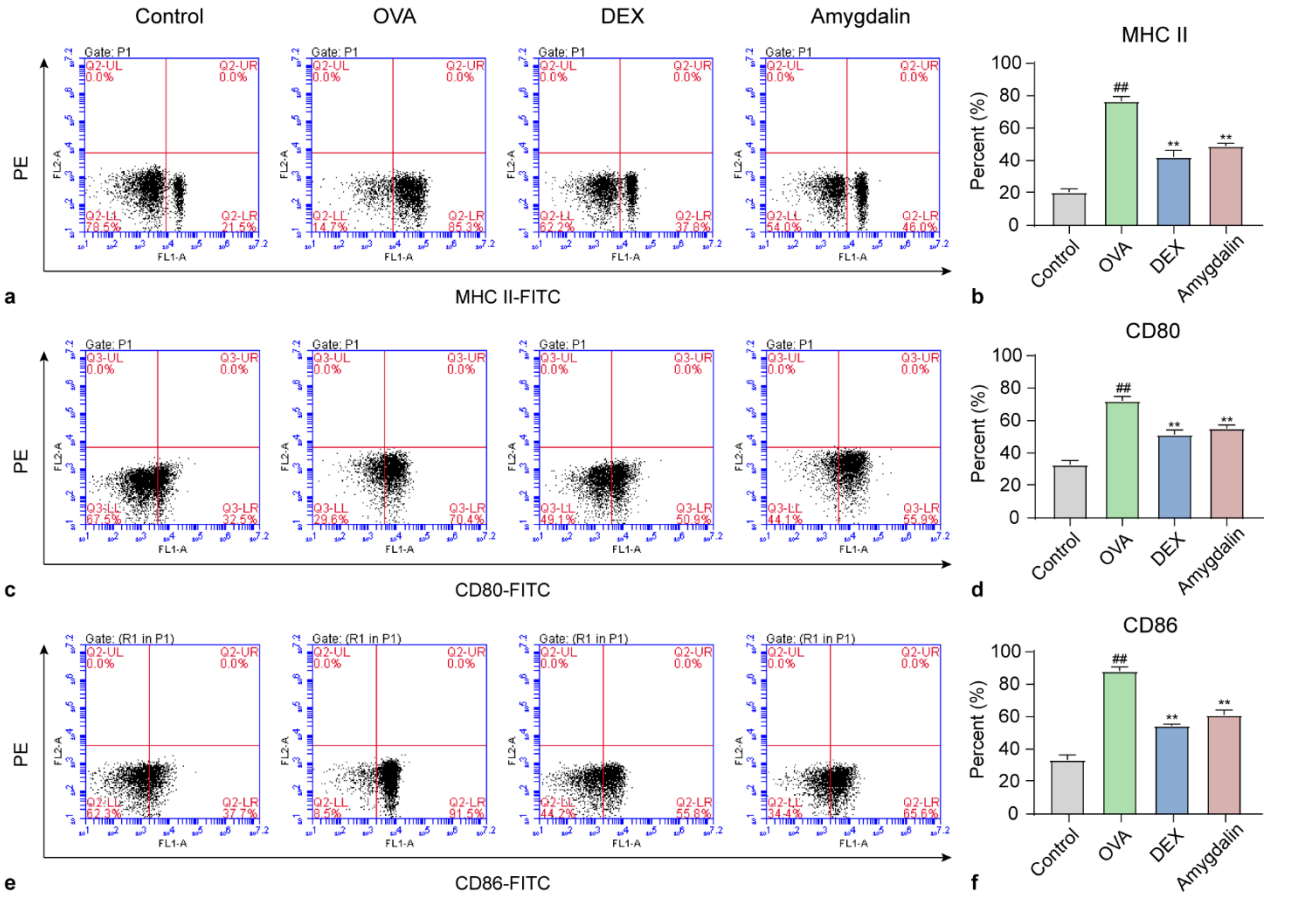

Figure 4. Amygdalin decreases the expression of cell-surface receptors in dendritic cells (DCs). Bone marrow-derived DCs were obtained from the 4 mouse groups. The expression of major histocompatibility complex class II (MHC II) (A), the cluster of differentiation (CD)80 (B), and CD86 (C) on the DC surface was assessed via flow cytometry. ## or ** p < 0.01; # *vs*. Control group; * *vs*. ovalbumin (OVA) group.

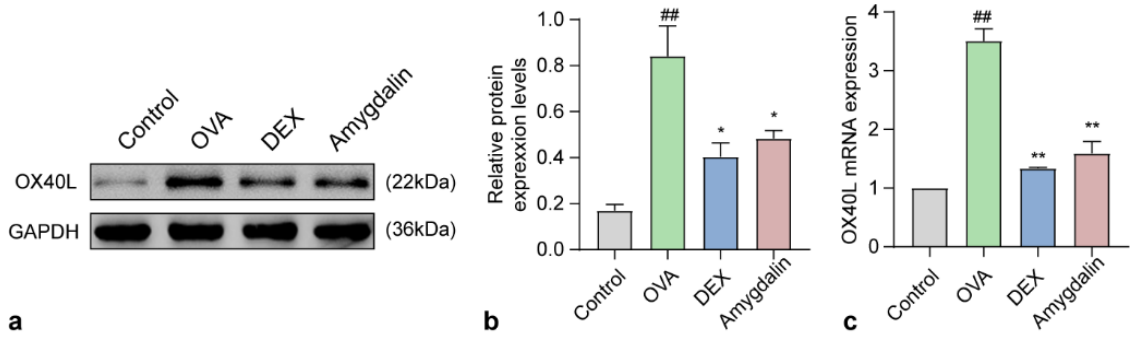

Figure 5. Amygdalin counteracts the effects on OX40L expression in dendritic cells (DCs) in ovalbumin (OVA)-sensitized mice. (A and B) OX40L expression in DCs derived from bone marrow was accomplished using western blotting, with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) employed as the internal control. c The mRNA levels of thymic stromal lymphopoietin *(TSLP)* in DCs were measured via quantitative real-time polymerase chain reaction (PCR). ## or ** $p<0.01$; # *vs*. Control group; * *vs*. OVA group.

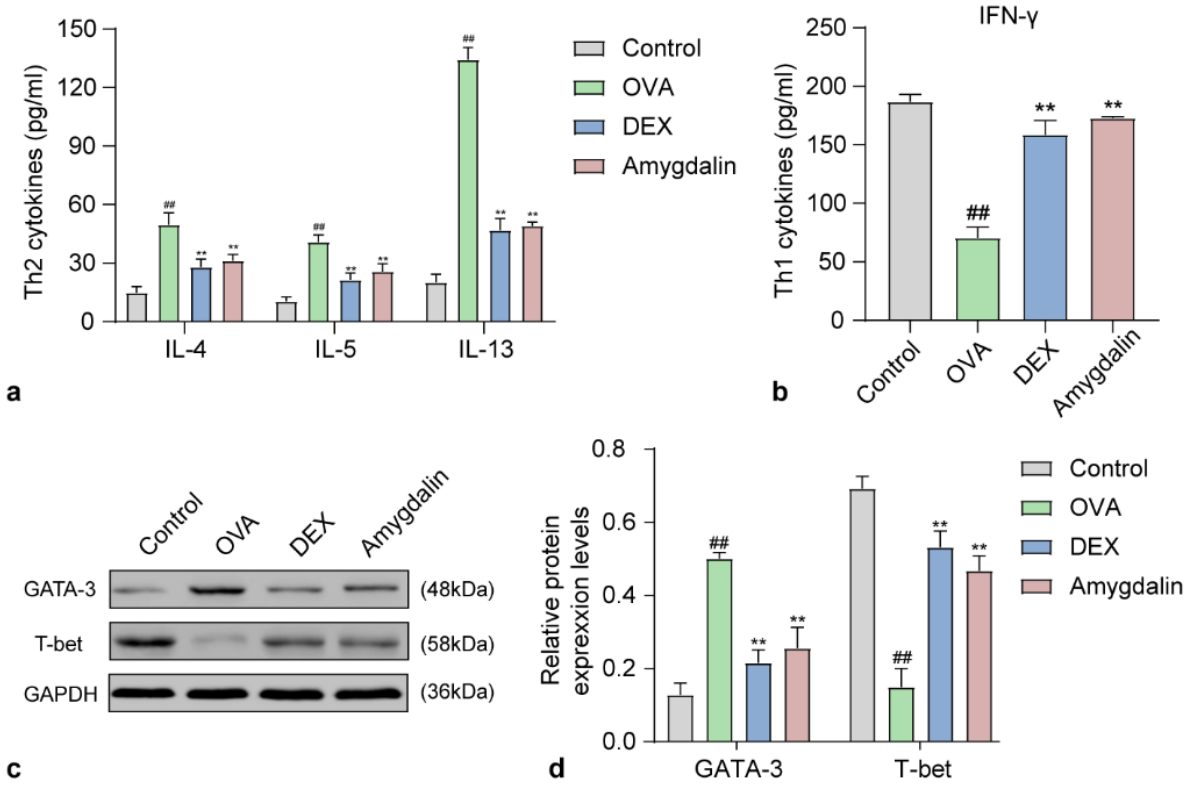

Figure 6. Effect of amygdalin on the T helper (Th)1/Th2 balance in ovalbumin (OVA)-sensitized mice. (A) Th2 cytokines levels (interleukin (IL)-4, IL-5, and IL-13), b Th1 cytokines levels (interferon (IFN)-γ) in bronchoalveolar lavage fluid (BALF) were determined via enzyme-linked immunosorbent assay (ELISA). c, d Western blotting analysis of GATA-3 and T-bet protein expression in lung tissues. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. ## or ** $p<0.01$; # *vs*. Control group; * *vs*. OVA group.

DISCUSSION

Asthma is a common chronic inflammatory disorder among the pediatric population. Over the years, there has been a steady increase in its prevalence.[20] Although various treatments are available, new drugs for improving inflammation must be actively explored.[21,22] Herein, we demonstrated that amygdalin, derived from the Chinese raw bitter almond, a traditional herbal medicine, could ameliorate allergic asthma in OVA-induced mouse models. Results showed that amygdalin attenuated airway inflammation through the TSLP–DC–OX40L axis and further regulated Th1/Th2 balance. Thus, amygdalin is a potential therapeutic agent for asthma.

Airway obstruction, primarily caused by persistent airway inflammation, is the principal characteristic of asthma.[5,23] We generated an allergic asthma mouse model via OVA sensitization. Airway inflammation in asthma is distinguished by infiltration and activation of various immune cells, including DCs, eosinophils, neutrophils, and lymphocytes.[24] Our findings demonstrate that amygdalin effectively mitigates airway inflammation in mice with OVA-induced allergic asthma.

TSLP is a cytokine most commonly found in the lungs, skin, and intestine.[25] Furthermore, it also assumes a substantial role in the development of allergic diseases.[26] TSLP is believed to play a role, at least in part, in various aspects of asthma pathophysiology, including increased airway sensitivity, excessive mucus generation, and structural changes to the airways. TSLP operates by engaging pro-inflammatory pathways that encompass cytokines like IL-4, IL-5, and IL-13.[27,28] Our study provided evidence that amygdalin effectively decreases TSLP expression in the airways of mice with allergic asthma. We hypothesized that amygdalin might modulate OVA-induced asthma by regulating TSLP expression. Given its central role in the initial stages of the inflammatory process, TSLP is a promising target for potential therapeutic interventions.[27]

Allergic asthma can be associated with TSLP overexpression to promote the activation of $CD4^+$ T lymphocytes by DCs.[29] The critical aspect of the immune response to allergens involves the regulation of DC activation.[30] CD80 and CD86, MHCII costimulatory molecules present on DCs, contribute to the differentiation of Th0 into Th1 or Th2 cells. Additionally, they play a central role in the initiation and progression of airway primary immune responses and allergic reactions.[31,32] The expression of MHCII, CD80, and CD86 was reduced on the surface of DCs after amygdalin treatment. TSLP binds to TSLPR on the DC surface to activate MHCII, CD80, and CD86 expression on the surface at a high level, promoting Th0 cell differentiation into Th2 cells and resulting in increased release of inflammatory factors and airway inflammation aggravation. Here, we found that this might be obstructed by amygdalin.

OX40L is primarily expressed in mature DCs, activated B cells, and macrophages and is virtually not expressed in immature or non-activated states. TSLP directly activates DCs through MHCII and costimulatory molecule upregulation, enhancing OX40L expression, promoting cell survival, and inducing chemokine secretion.[33] OX40L combined with OX40 (the receptor of OX40L) can regulate the activation and expansion of native $CD4^+$ T cells into Th2 cells.[34] Amygdalin downregulated OX40L expression at both protein and mRNA levels. Collectively, amygdalin regulates the TSLP–DC–OX40L axis in an OVA-induced allergic mouse model.

Th1/Th2 cell imbalances are considered the key mechanism of asthma pathogenesis.[35] Th2 cells carry out these functions by secreting a range of cytokines, including IL-4, IL-5, and IL-13, whereas Th1 cells suppress Th2 immune responses by secreting IFN-γ.[36] High GATA3 (crucial regulator of Th2 cells) expression and low T-bet (selectively expressed in Th1 cells) expression indicate localized imbalance in Th1 and Th2 cell differentiation.[37] Here, IL-4, IL-5, and IL-13 levels in the BALF were significantly higher, whereas IFN-γ levels were lower in the model group. Meanwhile, GATA-3 protein levels were upregulated, and those of T-bet were downregulated. Amygdalin treatment reversed this pattern, thereby restoring Th1/Th2 immune imbalance and improving signs of asthma.

In summary, we showed that amygdalin affects the polarization of TSLP-mediated DCs among lymphocytes, which can slow the maturation of DCs and reduce the OX40L expression. These results suggest that amygdalin might regulate the Th1/Th2 balance through the TSLP–DC–OX40L axis to participate in pathogenesis and airway inflammation in asthma, providing an experimental and theoretical basis for asthma treatment. By targeting the TSLP pathway to ameliorate airway inflammation, amygdalin emerges as an innovative therapeutic strategy for the clinical management of asthma.

STATEMENT OF ETHICS

All animal experiments were approved by the Institutional Animal Care and Use Committee at the Shanghai Municipal Hospital of Traditional Chinese Medicine (No. 2022017).

FUNDING

This study was supported by the National Natural Science Foundation of China (No.'s 8227150026, 82074488, and 81874488) and the Shanghai Sailing Program (No. 20YF1446000).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

Not applicable.

REFERENCES

1. GBC Chronic Respiratory Disease Collaborators. Prevalence and attributable health burden of chronic respiratory diseases, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Respir Med. 2020;8(6):585-96.
2. de Vreede I, Haarman EG, Sprikkelman AB, van Aalderen WM. From knemometry to final adult height: inhaled corticosteroids and their effect on growth in childhood. Paediatr Respir Rev. 2013;14(2):107-11; quiz 11, 137-8.
3. Heffler E, Madeira LNG, Ferrando M, Puggioni F, Racca F, Malvezzi L, et al. Inhaled corticosteroids safety and adverse effects in patients with asthma. J Allergy Clin Immunol Pract. 2018;6(3):776-81.
4. Lindsay JT, Heaney LG. Nonadherence in difficult asthma- facts, myths, and a time to act. Patient Prefer Adherence. 2013;7:329-36.
5. Hammad H, Lambrecht BN. The basic immunology of asthma. Cell. 2021;184(6):1469-85.
6. Han X, Krempski JW, Nadeau K. Advances and novel developments in mechanisms of allergic inflammation. Allergy. 2020;75(12):3100-11.
7. Simpson EL, Parnes JR, She D, Crouch S, Rees W, Mo M, et al. Tezepelumab, an anti-thymic stromal lymphopoietin monoclonal antibody, in the treatment of moderate to severe atopic dermatitis: A randomized phase 2a clinical trial. J Am Acad Dermatol. 2019;80(4):1013-21.
8. Guttman-Yassky E, Pavel AB, Zhou L, Estrada YD, Zhang N, Xu H, et al. GBR 830, an anti-OX40, improves skin gene signatures and clinical scores in patients with atopic dermatitis. J Allergy Clin Immunol. 2019;144(2):482-93.
9. Morianos I, Semitekolou M. Dendritic cells: Critical regulators of allergic asthma. Int J Mol Sci. 2020;21(21):7930.
10. Corren J, Ziegler SF. TSLP: from allergy to cancer. Nat Immunol. 2019;20(12):1603-9.
11. Murakami-Satsutani N, Ito T, Nakanishi T, Inagaki N, Tanaka A, Vien PTX, et al. IL-33 Promotes the induction and maintenance of Th2 immune responses by enhancing the function of OX40 ligand. Allergol Int. 2014;63(3): 443-55.
12. Feng S, Zhang L, Bian XH, Luo Y, Qin GH, Shi RM. Role of the TSLP-DC-OX40L pathway in asthma pathogenesis and airway inflammation in mice. Biochem Cell Biol. 2018;96(3):306-16.
13. Nyanhanda T, Gould EM, Hurst RD. Plant-derived foods for the attenuation of allergic airway inflammation. Curr Pharm Des. 2014;20(6):869-78.
14. Coleman SL, Shaw OM. Progress in the understanding of the pathology of allergic asthma and the potential of fruit proanthocyanidins as modulators of airway inflammation. Food Funct. 2017;8(12):4315-24.
15. Piao X, Jiang SH, Wang JN, Wu J, Xu WC, Li LQ, et al. Pingchuan formula attenuates airway mucus hypersecretion via regulation of the PNEC-GABA-IL13-Muc5ac axis in asthmatic mice. Biomed Pharmacother. 2021;140:111746.
16. Shi J, Chen Q, Xu M, Xia Q, Zheng T, Teng J, et al. Recent updates and future perspectives about amygdalin as a potential anticancer agent: A review. Cancer Med. 2019;8(6):3004-11.
17. He XY, Wu LJ, Wang WX, Xie PJ, Chen YH, Wang F. Amygdalin - A pharmacological and toxicological review. J Ethnopharmacol. 2020;254:112717.
18. Si Z, Zhang B. Amygdalin attenuates airway epithelium apoptosis, inflammation, and epithelial-mesenchymal transition through restraining the TLR4/NF-κB signaling pathway on LPS-treated BEAS-2B bronchial epithelial cells. Int Arch Allergy Immunol. 2021;182(10):997-1007.
19. Xue Z, Zhang XG, Wu J, Xu WC, Li LQ, Liu F, et al. Effect of treatment with geraniol on ovalbumin-induced allergic asthma in mice. Ann Allergy Asthma Immunol. 2016;116(6):506-13.
20. Castro-Rodriguez JA, Saglani S, Rodriguez-Martinez CE, Oyarzun MA, Fleming L, Bush A. The relationship

between inflammation and remodeling in childhood asthma: A systematic review. Pediatr Pulmonol. 2018;53(6):824-35.
21. Janssen LJ, Killian K. Airway smooth muscle as a target of asthma therapy: history and new directions. Respir Res. 2006;7:123.
22. Lam M, Royce SG, Samuel CS, Bourke JE. Serelaxin as a novel therapeutic opposing fibrosis and contraction in lung diseases. Pharmacol Ther. 2018;187:61-70.
23. Lambrecht BN, Hammad H. The immunology of asthma. Nat Immunol. 2015;16(1):45-56.
24. Casaro M, Souza VR, Oliveira FA, Ferreira CM. OVA-induced allergic airway inflammation mouse model. Methods Mol Biol. 2019;1916:297-301.
25. Fujio K, Nosaka T, Kojima T, Kawashima T, Yahata T, Copeland NG, et al. Molecular cloning of a novel type 1 cytokine receptor similar to the common gamma chain. Blood. 2000;95(7):2204-10.
26. Kitajima M, Lee HC, Nakayama T, Ziegler SF. TSLP enhances the function of helper type 2 cells. Eur J Immunol. 2011;41(7):1862-71.
27. Gauvreau GM, Sehmi R, Ambrose CS, Griffiths JM. Thymic stromal lymphopoietin: its role and potential as a therapeutic target in asthma. Expert Opin Ther Targets. 2020;24(8):777-92.
28. West EE, Kashyap M, Leonard WJ. TSLP: A key regulator of asthma pathogenesis. Drug Discov Today Dis Mech. 2012;9(3-4):10.1016/j.ddmec.2012.09.003.
29. Ziegler SF. The role of thymic stromal lymphopoietin (TSLP) in allergic disorders. Curr Opin Immunol. 2010;22(6):795-9.
30. Boonpiyathad T, Sozener ZC, Satitsuksanoa P, Akdis CA. Immunologic mechanisms in asthma. Semin Immunol. 2019;46:101333.
31. Lombardi V, Singh AK, Akbari O. The role of costimulatory molecules in allergic disease and asthma. Int Arch Allergy Immunol. 2010;151(3):179-89.
32. Lofdahl JM, Wahlstrom J, Skold CM. Different inflammatory cell pattern and macrophage phenotype in chronic obstructive pulmonary disease patients, smokers and non-smokers. Clin Exp Immunol. 2006;145(3):428-37.
33. Liu YJ, Soumelis V, Watanabe N, Ito T, Wang YH, Malefyt Rde W, et al. TSLP: an epithelial cell cytokine that regulates T cell differentiation by conditioning dendritic cell maturation. Annu Rev Immunol. 2007;25:193-219.
34. Gu W, Guo S, Zhang J, Zhang X, Sun Z, Chen Z, et al. March1-overexpressed dendritic cells downregulate Th1/Th2 ratio in asthma through promoting OX40L. Int Immunopharmacol. 2022;103:108444.
35. Bartemes KR, Kephart GM, Fox SJ, Kita H. Enhanced innate type 2 immune response in peripheral blood from patients with asthma. J Allergy Clin Immunol. 2014;134(3):671-8.e4.
36. Russkamp D, Aguilar-Pimentel A, Alessandrini F, Gailus-Durner V, Fuchs H, Ohnmacht C, et al. IL-4 receptor alpha blockade prevents sensitization and alters acute and long-lasting effects of allergen-specific immunotherapy of murine allergic asthma. Allergy. 2019;74(8):1549-60.
37. Eifan AO, Furukido K, Dumitru A, Jacobson MR, Schmidt-Weber C, Banfield G, et al. Reduced T-bet in addition to enhanced STAT6 and GATA3 expressing T cells contribute to human allergen-induced late responses. Clin Exp Allergy. 2012;42(6):891-900.