Allergologia et immunopathologia
Sociedad Española de Inmunología Clínica, Alergología y Asma Pediátrica
www.all-imm.com

ORIGINAL ARTICLE

OPEN ACCESS

Knockdown of KLK13 has a protective effect against ovalbumin-induced asthma in mice

Siyu Wu[a], Chengcheng Yang[b]*, Lu Chen[a], Mei Li[c], Shuo Wang[a]

[a]*Department of Pathology, The Second Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang, China*
[b]*Department of Pneumology, The Second Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang, China*
[c]*Department of Geriatric, The Second Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang, China*

Received 20 August 2025; Accepted 10 October 2025
Available online 1 January 2026

KEYWORDS
airway inflammation;
airway remodeling;
asthma;
KLK13;
NF-κB;
oxidative stress

Abstract
Background: Asthma remains a critical global health concern impacting diverse age groups, with incidence rates rising continuously. Recent studies have identified elevated levels of kallikrein-related peptidase 13 (KLK13) in nasal lavage specimens of asthma patients; however, its functional mechanisms remain explored.
Methods: An experimental asthma model was established in mice through ovalbumin (OVA) sensitization with an $Al(OH)_3$ adjuvant. The role of KLK13 was evaluated using immunohistochemistry, hematoxylin-eosin, and Masson trichrome staining, pulmonary function testing, enzyme-linked immunosorbent serological assay (ELISA), biochemical measurements, and immunoblotting.
Results: OVA-challenged mice exhibited marked upregulation of KLK13 expression. Genetic silencing of KLK13 reduced airway hyperresponsiveness in the asthma model. Histological analysis showed that OVA exposure caused extensive peribronchial inflammatory cell infiltration, bronchial wall thickening with luminal narrowing, and pulmonary collagen accumulation, all of which were significantly improved by KLK13 knockdown. OVA administration also induced substantial increases in interleukin (IL)-4, IL-5, IL-13, and immunoglobulin E, while KLK13 silencing significantly attenuated these elevations. Biochemical assays revealed increased malonaldehyde content and reactive oxygen species generation, alongside decreased superoxide dismutase activity and glutathione peroxidase levels in OVA-exposed mice; these changes were effectively normalized by KLK13 knockdown. At the molecular level, KLK13 inhibition reduced phosphorylation of inhibitor of nuclear factor-κB (NF-κB) alpha and p65 subunits, thereby suppressing activation of NF-κB pathway in OVA-induced asthma.

***Corresponding author:** Chengcheng Yang, Department of Pneumology, The Second Affiliated Hospital of Harbin Medical University, No. 246, Xuefu Road, Nangang District, Harbin City, Heilongjiang Province 150081, China. *Email address*: chengcy0190@163.com

https://doi.org/10.15586/aei.v54i1.1517

Conclusion: KLK13 depletion ameliorates asthmatic pathology by reducing airway inflammation, preventing structural remodeling, and restoring redox balance, primarily through NF-κB signaling modulation.

Introduction

Asthma, a common chronic inflammatory disorder of the airways, is characterized by reversible airflow obstruction and recurrent respiratory symptoms, such as wheezing, persistent cough, dyspnea, and chest constriction.[1,2] Epidemiological studies indicate wide geographical variation in prevalence of asthma (1–29% globally), with approximately 300 million people currently affected and the daily mortality exceeding 1000 cases worldwide.[2] Asthmatic patients are at increased risk not only of respiratory complications but also of cardiovascular and cerebrovascular comorbidities, creating major healthcare burdens.[3-5] Inhaled corticosteroids, especially in combination with formoterol, remain a cornerstone of asthma therapy across all severity levels.[2] Additionally, monoclonal antibodies targeting immune pathways implicated in asthma pathogenesis have been shown to reduce exacerbation frequency, improve lung function, and enhance quality of life (QoL).[6] Currently, the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) have approved biologics such as benralizumab, dupilumab, mepolizumab, and omalizumab, which act on diverse pathways involving immunoglobulin E (IgE), interleukin (IL)-4, IL-5, IL-13, and the IL-5 receptor α.[7] However, their clinical use is limited due to adverse effects and high costs.[2,7] These limitations underscore the importance of advancing personalized therapeutic approaches and precision medicine through the identification of novel biological pathways and cellular mechanisms.

Human tissue kallikrein enzymes (KLKs) represent a family of 15 serine proteases widely distributed across human tissues. They are particularly enriched in the respiratory system, where they play important roles in chronic inflammatory diseases, infections, and pulmonary malignancies.[8] Kallikrein-related peptidase 13 (KLK13/KLK-L4), located on chromosome *19q13.3-q13.4*, consists of five coding exons and four introns. KLK13 shows tissue-specific expression in organs, such as the prostate, mammary glands, salivary glands, and testicular tissues.[9] Emerging evidence indicates that KLK13 participates in multiple pulmonary pathologies. For instance, its expression correlates with tumor stage, metastatic potential, and patient survival in pulmonary carcinoma.[10-12] KLK13 also facilitates ß-coronavirus replication by activating viral spike proteins and intensifies pulmonary inflammatory responses.[13] Its role as a proteolytic activator has been mechanistically confirmed in human coronavirus HKU1 infection.[14] Moreover, proteomic analyses of nasal lavage fluid have identified elevated KLK13 levels in asthma patients.[15] Despite these associations, the functional mechanisms of KLK13 in asthma remain poorly understood.

To address this gap, we employed an ovalbumin-induced murine asthma model to investigate the role of KLK13 in progression of the disease. Our findings demonstrate that KLK13 inhibition alleviates asthma by modulating nuclear factor-κB (NF-κB) signaling, thereby reducing pulmonary inflammation, preventing airway remodeling, and mitigating oxidative stress, highlighting its potential as a therapeutic target in management of the respiratory disease.

Materials and Methods

Animals

Female C57BL/6 mice aged 7–8 weeks (average body weight ~20 g) were obtained from Cyagen Biosciences (Jiangsu, China) and housed under controlled environmental conditions with a 12-h light/dark cycle. The animals received standardized laboratory feed and had continuous access to purified water throughout the study period. All experimental procedures conformed to the guidelines of the Guide for the Care and Use of Laboratory Animals and were approved by the Animal Research Ethics Committee of our hospital.[16]

Animal treatment

The chronic asthma model was established based on previously described protocols.[17] Mice were sensitized with intraperitoneal injections of ovalbumin (OVA) mixed with $Al(OH)_3$ adjuvant on days 0, 7, and 14. From day 21 to day 58, animals were exposed intermittently to 3% OVA aerosol to induce airway inflammation. The sensitization solution consisted of 20-μg OVA (01641, ≥97% purity; Sigma, St. Louis, MO, USA) dissolved in 200-μL phosphate-buffered saline (PBS, P1020; Solarbio, Beijing, China) and mixed with 2-mg $Al(OH)_3$ (239186; Sigma).

During the challenge phase, viral vectors carrying KLK13-targeting short hairpin RNA (shRNA) or non-targeting control sequences were administered via caudal vein injection in three fractional doses. Experimental cohorts (n = 6/group) included: control, OVA, OVA+short hairpin negative control (shNC), and OVA+shKLK13 groups. All asthma groups underwent OVA-$Al(OH)_3$ sensitization, followed by intravenous delivery of PBS (OVA group), control shRNA (OVA+shNC), or KLK13-specific shRNA (OVA+shKLK13). The control group received PBS throughout while otherwise following the same protocol. Airway hyperresponsiveness (AHR) was assessed by pulmonary function testing after final nasal provocation. Blood samples were collected for IgE measurement, and bronchoalveolar lavage fluid (BALF) was obtained by washing lung tissues with 1 ml of 1×Hank's

balanced salt solution (HBSS, H1025; Solarbio). Parallel lung tissues were collected for histological, biochemical, and molecular analyses.

AHR measurement

Respiratory function was evaluated by whole-body plethysmography (WBP-4MR; TOW, China). Mice were placed in measurement chambers and acclimated for 5 min before exposure to methacholine aerosols at concentrations of 0, 50, and 100 mg/mL. Airway reactivity was quantified by continuously recording enhanced pause (Penh) values during the challenge.

Pathological staining

Lung tissues were fixed in 4% paraformaldehyde, dehydrated in graded ethanol, and embedded in paraffin (YA0011; Solarbio). Sections of 5-μm thickness were cut and stained with hematoxylin and eosin (H&E, G1120; Solarbio) or Masson staining (G1340; Solarbio). Slides were examined with an Olympus microscope, and digital images were analyzed using Image-Pro Plus 6.0 (Media Cybernetics, USA).

Immunohistochemistry

Paraffin sections underwent antigen retrieval in sodium citrate buffer (pH 6.0, P0081; Beyotime, Shanghai, China) at 94°C for 15 min, followed by blocking with 1% bovine serum albumin (BSA, ST2249; Beyotime) for 1 h at room temperature. Sections were incubated overnight at 4°C with primary antibodies against KLK13 (1:50, IPDX08469; IPODIX, Wuhan, China), then with goat anti-rabbit immunoglobulin G (IgG) H&L (horseradish peroxidase [HRP]) secondary antibody (1:1000, ab6721; Abcam, UK) at 37°C for 30 min. Hematoxylin counterstaining (G1080; Solarbio) was performed before visualization with an Olympus light microscope.

Enzyme-linked immunosorbent serological assay (ELISA)

Cytokine levels were determined using ELISA kits from Beyotime for IL-4 (PI612), IL-5 (PI620), IL-13 (PI539), and IgE (PI476). Absorbance was measured at 450 nm with a Thermo Fisher Scientific microplate reader (Waltham, MA, USA).

Colorimetric assays

Lung tissue concentrations of malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) were measured using commercial kits from Sangon Biotech (D799761, D799593, D799613, respectively; Shanghai, China). Reactive oxygen species (ROS) were quantified using the ROS assay kit (E004-1-1; Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions.

Western blotting analysis

Lung specimens were homogenized in radioimmunoprecipitation assay (RIPA) lysis buffer (20101ES60; YEASEN, Shanghai, China), and protein concentrations were measured with the bicinchoninic acid (BCA) protein assay kit (20201ES76; YEASEN). Equal amounts of protein (20 μg) were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes (36126ES03; YEASEN). After blocking with 5% BSA for 1 h, membranes were incubated overnight at 4°C with primary antibodies against KLK13 (1:1000, IPDX08469; IPODIX), p65 (1:1000, ab16502; Abcam), phosphorylated p65 (p-p65, 1:1000, ab76302; Abcam), nuclear factor-κB alpha (IκBα) (1:5000, ab97783; Thermo Fisher Scientific), phosphorylated IκBα (p-IκBα; 1:10,000, ab133462; Abcam), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH, 1:1000, ab263962; Abcam). Membranes were washed and incubated with HRP-conjugated goat anti-rabbit IgG (1:10,000, ab6721; Abcam) for 1 h at room temperature. Protein signals were visualized using enhanced chemiluminescence detection. The BeyoECL Plus detection kit (P0018S; Beyotime) was employed for protein analysis, with grayscale quantification performed through the Image-ProPlus analytical software (Media Cybernetics, Rockville, MD).

Statistical analysis

Experimental results were expressed as mean ± standard deviation (SD). Statistical analyses were conducted with SPSS 20.0 (IBM Corp., Armonk, NY, USA). Differences between two groups were evaluated using Student's *t*-test, while comparisons among multiple groups were performed by one-way ANOVA followed by Tukey's *post hoc* test. Statistical significance threshold was established at $P < 0.05$ for all experimental determinations.

Results

KLK13 was upregulated in OVA-induced asthmatic mice

To investigate the role of KLK13 in asthma, its expression was first examined in OVA-induced mice. Both immunoblotting and immunohistochemistry demonstrated a pronounced increase in KLK13 expression in OVA-treated mice, compared to controls (Figures 1A and B). These findings confirmed that KLK13 was upregulated in the asthmatic mice.

KLK13 knockdown alleviated histopathological changes in asthmatic mice

To further explore the role of KLK13, its expression was suppressed using lentiviral vectors carrying shKLK13. KLK13 expression was markedly reduced in OVA-induced mice following lentiviral delivery, confirming efficient knockdown (Figure 2A). AHR, which was significantly elevated

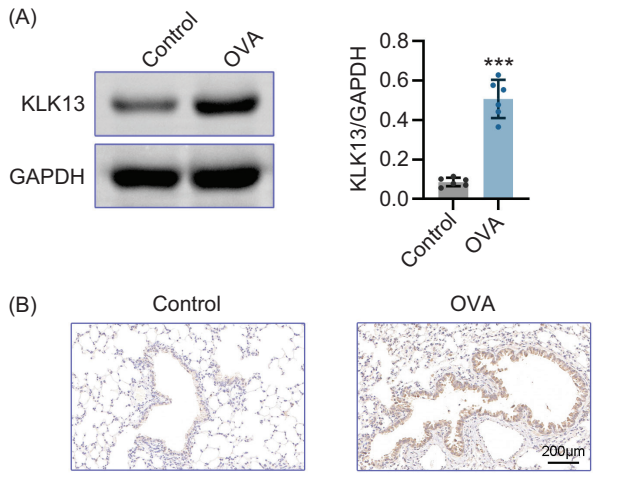

Figure 1 KLK13 was upregulated in OVA-induced mice. (A) The KLK13 expression was examined by Western blot analysis. Protein expressions were normalized to GAPDH. (B) The KLK13 level was examined by immunohistochemistry. Scale bar = 200 μm. ***P < 0.001 vs. the control.

in OVA-treated mice, was partially improved after KLK13 silencing (Figure 2B). Histological analysis showed that OVA exposure led to dense inflammatory cell infiltration around small airways, accompanied by bronchial wall thickening and luminal constriction. These pathological alterations were substantially alleviated by KLK13 knockdown (Figure 2C). Masson staining further revealed enhanced collagen deposition around the airway epithelium in OVA-induced mice that was significantly reduced following shKLK13 treatment (Figure 2D). Collectively, KLK13 silencing mitigated airway inflammation, remodeling, and hyperresponsiveness in asthmatic mice.

KLK13 knockdown reduced T helper 2 (Th2) cell immune response in asthmatic mice

To study the role of KLK13 on Th2-mediated immunity, levels of Th2-associated cytokines were measured. OVA exposure markedly increased the concentrations of IL-4, IL-5, IL-13, and IgE, whereas KLK13 knockdown significantly counteracted these elevations (Figures 3A and B). These results indicate that KLK13 silencing attenuated the Th2 immune response in asthmatic mice.

KLK13 knockdown mitigated oxidative stress in asthmatic mice

Oxidative stress markers were evaluated to determine the contribution of KLK13 to redox imbalance in asthma. OVA-induced mice exhibited significantly elevated MDA and ROS levels, accompanied by decreased SOD activity and GSH-Px levels (Figures 4A and B). Importantly, KLK13 knockdown effectively reversed these changes, restoring redox homeostasis. Thus, KLK13 silencing alleviated oxidative stress in asthmatic mice.

KLK13 knockdown inhibited activation of the NF-κB pathway

Mechanistically, OVA stimulation led to increased phosphorylation of p65 and IκBα while reducing total IκBα expression. These alterations were significantly neutralized following KLK13 knockdown (Figure 5). Taken together, these findings demonstrate that KLK13 silencing suppressed activation of the NF-κB signaling pathway in asthma model.

Discussion

In this study, OVA administration triggered a significant increase in KLK13 expression. Genetic silencing of KLK13 reduced pathological changes, suppressed Th2-mediated immune responses, and alleviated oxidative stress in asthmatic mice. Mechanistic analysis further showed that KLK13 knockdown inhibited activation of the NF-κB pathway in OVA-challenged animals. Together, these findings indicate that KLK13 downregulation mitigates airway inflammation, structural remodeling, and oxidative damage in asthma, with NF-κB signaling acting as a key regulatory mechanism.

KLK13 is reported to be aberrantly expressed in several pathological contexts, including tumors[10,18] and infections.[14] Consistent with previous proteomic analyses in nasal lavage samples from asthma patients,[15] our results revealed elevated KLK13 levels in OVA-induced mice, suggesting its involvement in asthma pathogenesis. Experimental suppression of KLK13 significantly alleviated AHR, in line with earlier studies demonstrating similar protective effects of gene knockdown in asthma models.[19-21] OVA-exposed mice in our study also displayed classic histopathological features, such as peribronchial inflammatory cell infiltration, bronchial wall thickening with luminal narrowing, and enhanced collagen accumulation, which were notably improved following KLK13 knockdown.[22] Previous studies demonstrated that inhibition of inflammatory cell migration played a therapeutic role in asthma; for example, deletion of tartrate-resistant acid phosphatase 5 (TRAP) reduced eosinophil migration in allergic asthma,[23] while inhibition of myristoylated alanine-rich C kinase substrate (MARCKS) phosphorylation suppressed dendritic cell migration in acute asthma models.[24] Our results extend this concept by showing that KLK13 inhibition reduced airway inflammation and remodeling. Evidence indicates that both airway remodeling and inflammatory cell infiltration are major drivers of AHR.[25] Structural remodeling—including airway wall thickening, subepithelial fibrosis, and collagen deposition—is strongly associated with impaired lung function.[26] Notably, KLK family members, such as KLK5, also have been implicated in airway remodeling in chronic obstructive pulmonary disease (COPD).[27] Collectively, our findings suggest that KLK13 silencing prevents both structural airway abnormalities and excessive bronchoconstriction in OVA-induced asthma.

Asthma is a complex disorder with heterogeneous clinical phenotypes, yet persistent airway inflammation remains its hallmark.[28] Pathogenesis is largely driven by

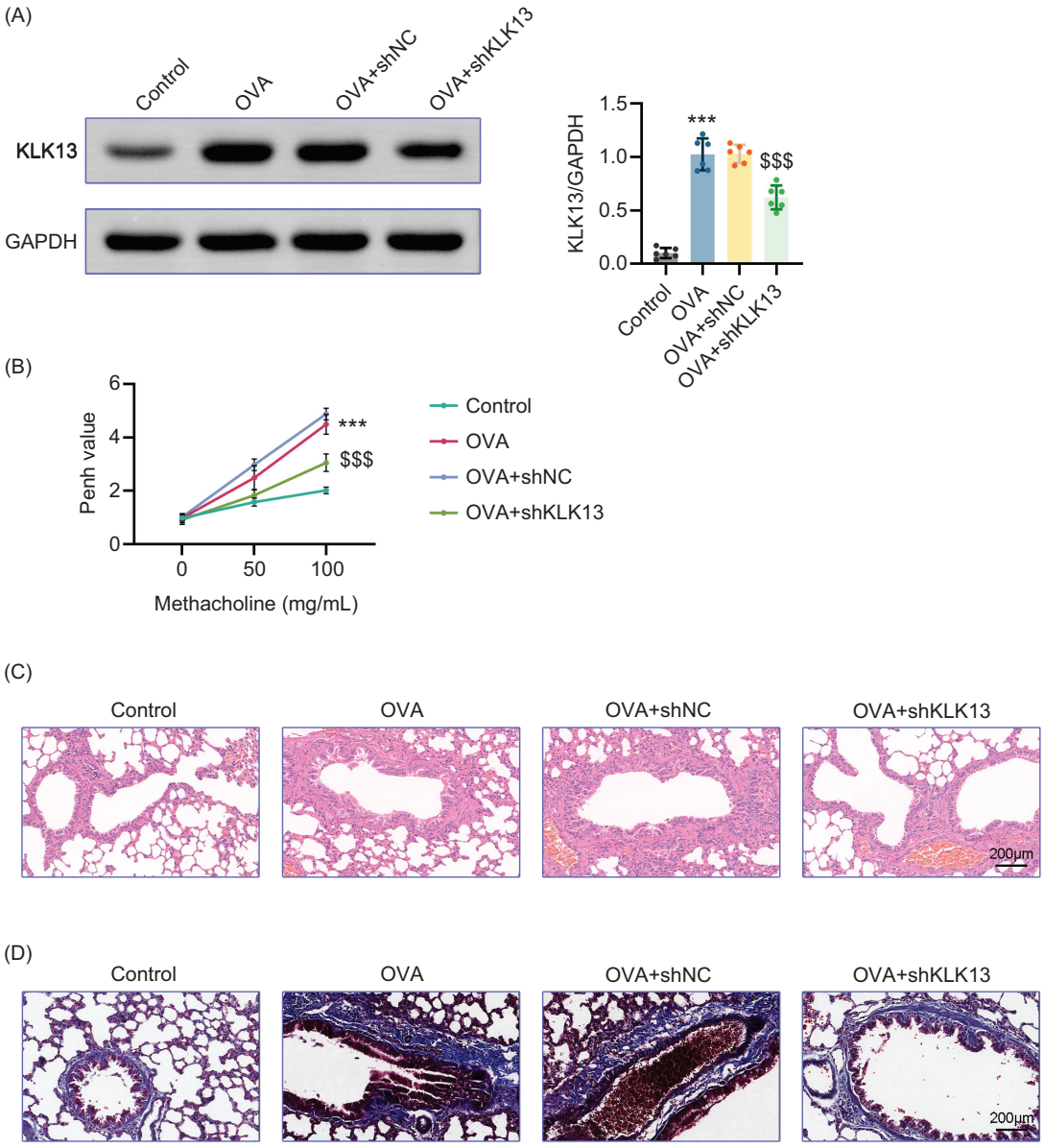

Figure 2 KLK13 knockdown ameliorated AHR and lung histopathological symptoms in OVA-induced mice. (A) The KLK13 expression was examined by Western blot analysis following the injection of LV-packed shKLK13. The protein expressions were normalized to GAPDH. (B) Measurement of AHR. (C and D) Lung tissues were conducted by hematoxylin and eosin (H&E) and Masson staining. Scale bar = 200 μm. $^{***}P < 0.001$ vs. the control; $^{\$\$\$}P < 0.001$ vs. OVA+shNC.

type 2 inflammatory pathways mediated by key immune components, including Th2 lymphocytes, group 2 innate lymphoid cells (ILC2s), dendritic cells, eosinophilic granulocytes, and respiratory epithelial cells.[29,30] Environmental allergens induce differentiation of naive Th0 lymphocytes into Th2 cells, promoting the release of IL-4, IL-5, and IL-13.[31] IL-4 is a critical mediator of B-cell activation and IgE synthesis,[32] IL-5 regulates eosinophil maturation and migration,[33] while IL-13 contributes to AHR, mucus secretion, and shares overlapping roles with IL-4.[34] Elevated eosinophilia and increased IL-4, IL-5, and IL-13 are hallmarks of Th2-high asthma,[35] and higher serum concentrations of these cytokines have been consistently reported in patients.[36] In our study, OVA challenge markedly increased IL-4, IL-5, IL-13, and IgE levels, which were significantly reduced by KLK13 knockdown, underscoring the contribution of KLK13 to Th2-mediated airway inflammation, aligning with earlier studies.[20,21]

Oxidative stress is increasingly recognized as a central pathogenic factor in asthma and other chronic diseases.[37-41] Excessive oxidative burden amplifies airway inflammation, promotes structural remodeling, drives mucus hypersecretion, and contributes to lung tissue injury.[42,43] Consistent with earlier reports,[21,22] OVA exposure in our study led to increased MDA and ROS levels, along with reduced antioxidant defenses (SOD and GSH-Px). MDA and ROS serve as key indicators of oxidative stress,[44] while SOD and GSH are vital antioxidant enzymes known to decline in patients with

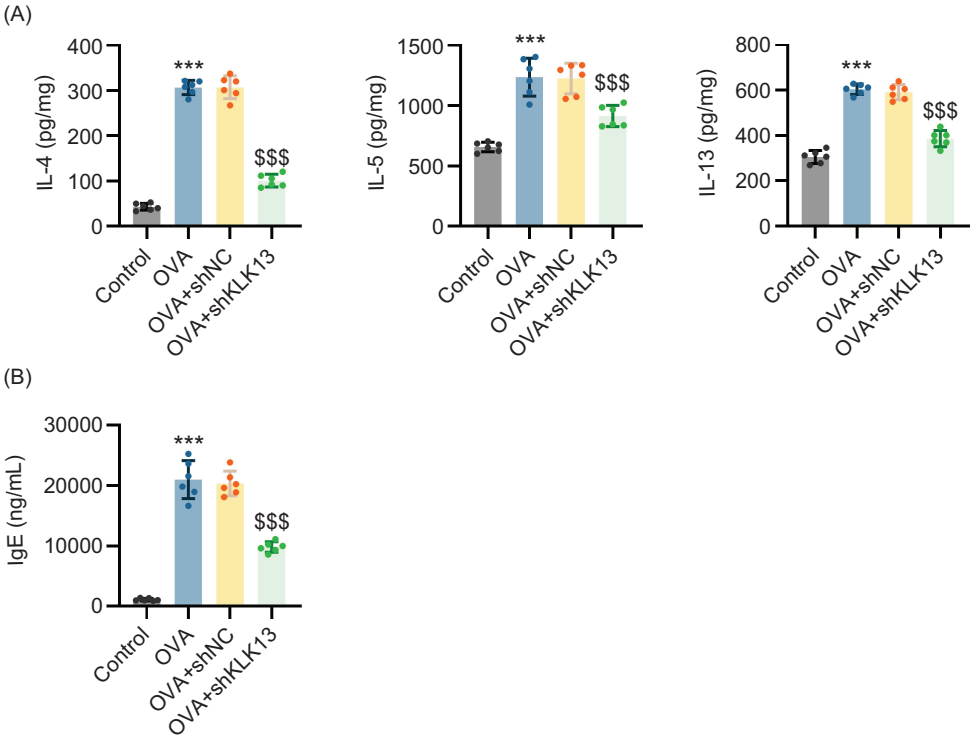

Figure 3 KLK13 knockdown attenuated the inflammatory factors release in OVA-induced mice. (A) The concentrations of IL-4, IL-5, and IL-13 in BALF were examined by ELISA. (B) The concentrations of IgE in sera were examined by ELISA. $^{***}P < 0.001$ vs. the control; $^{\$\$\$}P < 0.001$ vs. OVA+shNC.

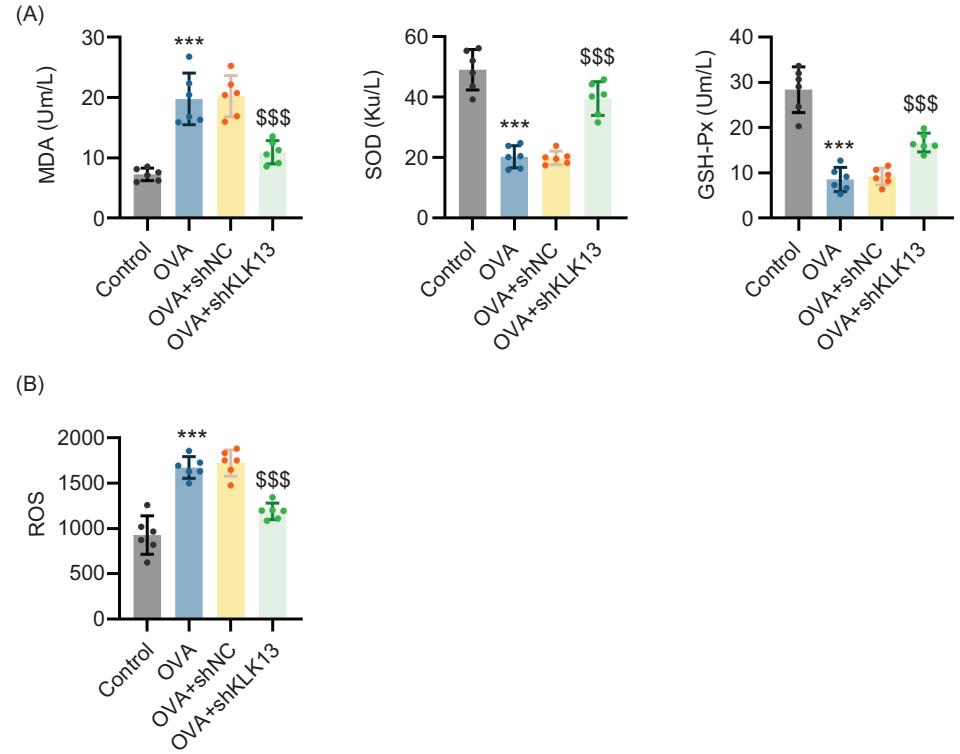

Figure 4 KLK13 knockdown reduced oxidative stress in OVA-induced mice. (A and B) The contents of MDA, SOD, GSH-Px, and ROS in lung tissues were determined by biochemical examination. $^{***}P < 0.001$ vs. the control; $^{\$\$\$}P < 0.001$ vs. OVA+shNC.

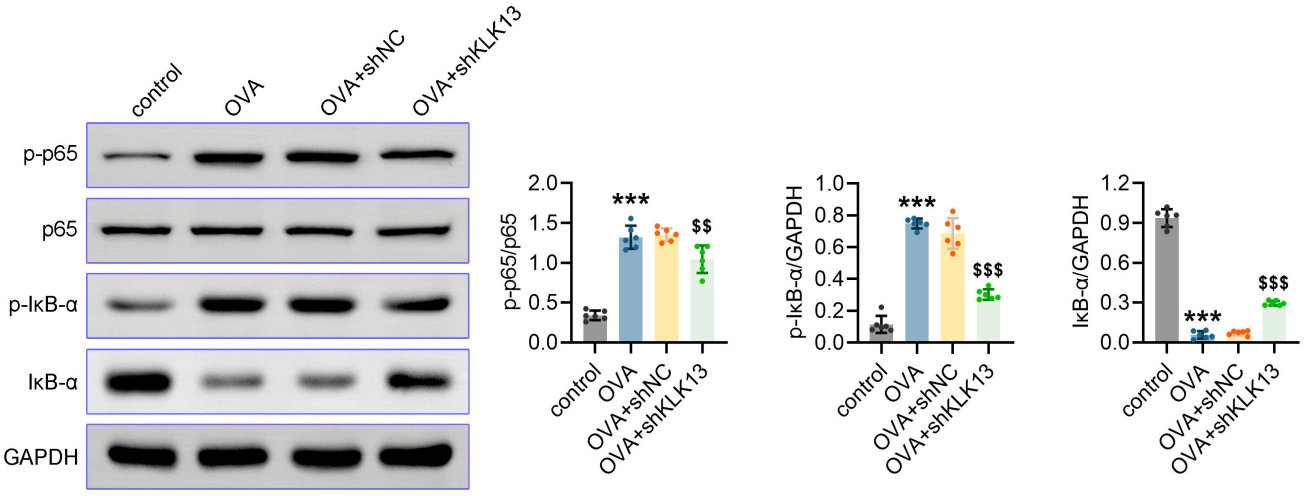

Figure 5 KLK13 knockdown suppressed the activation of NF-κB signaling in OVA-induced mice. The relative protein expression of p65, p-p65, IκB-α, and p-IκB-α was examined by Western blot analysis. Protein expressions were normalized to GAPDH. ***P < 0.001 vs. the control; $$$P < 0.001 vs. OVA+shNC.

allergic asthma.[42] Importantly, KLK13 knockdown restored oxidative balance, highlighting its role in regulating redox homeostasis during progression of asthma.

The NF-κB pathway is a master regulator of inflammatory responses and is implicated in multiple chronic diseases.[45,46] Under resting conditions, NF-κB dimers (p65/p50) are sequestered in the cytoplasm through binding with IκBα. Upon stimulation, IκBα is phosphorylated and degraded, allowing NF-κB to translocate into the nucleus and activate transcription of pro-inflammatory mediators.[47,48] This pathway orchestrates both cytokine production and eosinophil recruitment, key features of allergic asthma. Enhanced NF-κB activation is observed in bronchial biopsies from severe asthma patients,[49] and pharmacological inhibition of this pathway is shown to alleviate airway inflammation in experimental models.[50,51] In our study, OVA exposure significantly increased phosphorylation of p65 and IκBα while decreasing total IκBα protein, whereas KLK13 knockdown effectively reversed these changes. These results confirmed that KLK13 silencing suppresses NF-κB pathway activation in asthma.

Conclusion

Suppression of KLK13 alleviated airway hyperreactivity, inflammatory infiltration, and oxidative stress in OVA-induced asthma—the effects that were strongly associated with inhibition of NF-κB signaling. Nevertheless, the exact contribution of NF-κB requires further verification through targeted pathway activation studies. Additional preclinical investigations are warranted to assess therapeutic feasibility and optimize clinical translation strategies. Our findings highlight KLK13 as a promising therapeutic target for asthma management and establish a framework for its potential clinical development.

Data Availability Statement

The datasets used and/or analyzed in the present study are available from the corresponding author on reasonable request.

Ethical Approval

Ethical approval was obtained from the Ethics Committee of the Second Affiliated Hospital of Harbin Medical University (Approval No. SYDW2025-110).

The animal experiment complied with the ARRIVE guidelines and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Author's Contribution

All authors contributed to the study's conception and design. Material preparation and the experiments were performed by Siyu Wu. Data collection and analysis were performed by Chengcheng Yang, Lu Chen, and Mei Li. The first draft of the manuscript was written by Shuo Wang, and all authors commented on the previous versions of the manuscript. All authors had read and approved the final manuscript.

Conflict of Interests

The authors declared that there was no conflict of interests.

Funding

Not applicable.

References

1. Porsbjerg C, Melén E, Lehtimäki L, Shaw D. Asthma. Lancet. 2023;401(10379):858-73. https://doi.org/10.1016/S0140-6736(22)02125-0
2. Global Initiative for Asthma. Global strategy for asthma management and prevention, 2025 [internet]. [cited May 2025]. Available from: www.ginasthma.org.
3. Zhang B, Li ZF, An ZY, Zhang L, Wang JY, Hao MD, et al. Association between asthma and all-cause mortality and cardiovascular disease morbidity and mortality: A meta-analysis of cohort studies. Front Cardiovasc Med. 2022;9:861798. https://doi.org/10.3389/fcvm.2022.861798
4. Naqvi H, Searles CD. Association between asthma and cardiovascular disease among a United States representative population. Int J Cardiol Cardiovasc Risk Prev. 2025;26:200451. https://doi.org/10.1016/j.ijcrp.2025.200451
5. Jinghua S, Gaonian Z, Su J. Relationship between allergic asthma and cerebrovascular accident: Allergic asthma can increase recurrence of stroke. Postepy Dermatol Alergol. 2025;42(2):150-55. https://doi.org/10.5114/ada.2024.145458
6. Bacharier LB, Jackson DJ. Biologics in the treatment of asthma in children and adolescents. J Allergy Clin Immunol. 2023;151(3):581-89. https://doi.org/10.1016/j.jaci.2023.01.002
7. Wirthgen E, Quickert S, Weitzel J, Salewski B, Ballmann M. Safety of biologics for the treatment of asthma in children and adolescents: A systematic review. Eur Respir Rev. 2025;34(176):240269. https://doi.org/10.1183/16000617.0269-2024
8. Lenga Ma Bonda W, Iochmann S, Magnen M, Courty Y, Reverdiau P. Kallikrein-related peptidases in lung diseases. Biol Chem. 2018;399(9):959-71. https://doi.org/10.1515/hsz-2018-0114
9. Yousef GM, Chang A, Diamandis EP. Identification and characterization of KLK-L4, a new Kallikrein-like gene that appears to be downregulated in breast cancer tissues. J Biol Chem. 2000;275(16):11891-98. https://doi.org/10.1074/jbc.275.16.11891
10. Sumiya R, Yamada K, Hagiwara T, Nagasaka S, Miyazaki H, Igari T, et al. Kallikrein-related peptidase 13 expression and clinicopathological features in lung squamous cell carcinoma. Mol Clin Oncol. 2023;19(2):64. https://doi.org/10.3892/mco.2023.2660
11. Wang Y, Tan TZ, Wang LQ, Zhang K. LncRNA WT-AS inhibits metastatic ability of non-small cell lung cancer by regulating KLK13. Eur Rev Med Pharmacol Sci. 2020;24(18):9429-37.
12. Gueugnon F, Barascu A, Mavridis K, Petit-Courty A, Marchand-Adam S, Gissot V, et al. Kallikrein-related peptidase 13: An independent indicator of favorable prognosis for patients with nonsmall cell lung cancer. Tumour Biol. 2015;36(7):4979-86. https://doi.org/10.1007/s13277-015-3148-1
13. Kim H, Kang Y, Kim S, Park D, Heo SY, Yoo JS, et al. The host protease KLK5 primes and activates spike proteins to promote human betacoronavirus replication and lung inflammation. Sci Signal. 2024;17(850):eadn3785. https://doi.org/10.1126/scisignal.adn3785
14. Milewska A, Falkowski K, Kulczycka M, Bielecka E, Naskalska A, Mak P, et al. Kallikrein 13 serves as a priming protease during infection by the human coronavirus HKU1. Sci Signal. 2020;13(659):eaba9902. https://doi.org/10.1126/scisignal.aba9902
15. Chen M, Ge Y, Zhang W, Wu P, Cao C. Nasal lavage fluid proteomics reveals potential biomarkers of asthma associated with disease control. J Asthma Allergy. 2024;17:449-62. https://doi.org/10.2147/JAA.S461138
16. National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. Washington, DC: National Academies of Sciences Press (US), 2011. PMID: 21595115.
17. Zeng R, Zhang D, Zhang J, Pan Y, Liu X, Qi Q, et al. Targeting lysyl oxidase like 2 attenuates OVA-induced airway remodeling partly via the AKT signaling pathway. Respir Res. 2024;25(1):230. https://doi.org/10.1186/s12931-024-02811-4
18. Lin Q, Mao W, Wu Q, He X, Li S, Fan Y, et al. Downregulation of KLK13 promotes the invasiveness and metastasis of oesophageal squamous cell carcinoma. Biomed Pharmacother. 2017;96:1008-15. https://doi.org/10.1016/j.biopha.2017.11.129
19. Lu C, Zhang B, Xu T, Zhang W, Bai B, Xiao Z, et al. Piperlongumine reduces ovalbumin-induced asthma and airway inflammation by regulating nuclear factor-κB activation. Int J Mol Med. 2019;44(5):1855-65. https://doi.org/10.3892/ijmm.2019.4322
20. Jia S, Guo P, Lu J, Huang X, Deng L, Jin Y, et al. Curcumol ameliorates lung inflammation and airway remodeling via inhibiting the abnormal activation of the Wnt/ß-catenin pathway in chronic asthmatic mice. Drug Des Devel Ther. 2021;15:2641-51. https://doi.org/10.2147/DDDT.S292642
21. Zhang N, Lu HT, Zhang RJ, Sun XJ. Protective effects of methane-rich saline on mice with allergic asthma by inhibiting inflammatory response, oxidative stress and apoptosis. J Zhejiang Univ Sci B. 2019;20(10):828-37. https://doi.org/10.1631/jzus.B1900195
22. Wang Z, Zhang H, Sun X, Ren L. The protective role of vitamin D3 in a murine model of asthma via the suppression of TGF-ß/Smad signaling and activation of the Nrf2/HO-1 pathway. Mol Med Rep. 2016;14(3):2389-96. https://doi.org/10.3892/mmr.2016.5563
23. Padra M, Bergwik J, Adler A, Marcoux G, Bhongir RKV, Papareddy P, et al. Tartrate-resistant acid phosphatase 5 (TRAP5) promotes eosinophil migration during allergic asthma. Am J Respir Cell Mol Biol. 2025;73(4):612-22. https://doi.org/10.1165/rcmb.2024-0304OC
24. Lee CC, Chen CH, Kenyon NJ, Wang CN, Tsai HC, Chiu CL, et al. Inhibition of MARCKS phosphorylation attenuates of dendritic cell migration in a murine model of acute asthma. Eur J Pharmacol. 2024;176867. https://doi.org/10.1016/j.ejphar.2024.176867
25. Gon Y, Hashimoto S. Role of airway epithelial barrier dysfunction in pathogenesis of asthma. Allergol Int. 2018;67(1):12-7. https://doi.org/10.1016/j.alit.2017.08.011
26. Chen YF, Huang G, Wang YM, Cheng M, Zhu FF, Zhong JN, et al. Exchange protein directly activated by cAMP (Epac) protects against airway inflammation and airway remodeling in asthmatic mice. Respir Res. 2019;20(1):285. https://doi.org/10.1186/s12931-019-1260-2
27. Lenga Ma Bonda W, Lavergne M, Vasseur V, Brisson L, Roger S, Legras A, et al. Kallikrein-related peptidase 5 contributes to the remodeling and repair of bronchial epithelium. Faseb J. 2021;35(10):e21838. https://doi.org/10.1096/fj.202002649R
28. Willis-Owen SAG, Cookson WOC, Moffatt MF. The genetics and genomics of asthma. Annu Rev Genomics Hum Genet. 2018;19:223-46. https://doi.org/10.1146/annurev-genom-083117-021651
29. Lambrecht BN, Hammad H, Fahy JV. The cytokines of asthma. Immunity. 2019;50(4):975-91. https://doi.org/10.1016/j.immuni.2019.03.018
30. Pritam P, Manna S, Sahu A, Swain SS, Ramchandani S, Bissoyi S, et al. Eosinophil: A central player in modulating pathological complexity in asthma. Allergol Immunopathol (Madr). 2021;49(2):191-207. https://doi.org/10.15586/aei.v49i2.50
31. Kidd P. Th1/Th2 balance: The hypothesis, its limitations, and implications for health and disease. Altern Med Rev. 2003;8(3):223-46.
32. Steinke JW, Borish L. Th2 cytokines and asthma. Interleukin-4: Its role in the pathogenesis of asthma, and

targeting it for asthma treatment with interleukin-4 receptor antagonists. Respir Res. 2001;2(2):66–70. https://doi.org/10.1186/rr40
33. Foster PS, Hogan SP, Ramsay AJ, Matthaei KI, Young IG. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. J Exp Med. 1996;183(1):195–201. https://doi.org/10.1084/jem.183.1.195
34. Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, et al. Interleukin-13: Central mediator of allergic asthma. Science. 1998;282(5397):2258–61. https://doi.org/10.1126/science.282.5397.2258
35. Truyen E, Coteur L, Dilissen E, Overbergh L, Dupont LJ, Ceuppens JL, et al. Evaluation of airway inflammation by quantitative Th1/Th2 cytokine mRNA measurement in sputum of asthma patients. Thorax. 2006;61(3):202–8. https://doi.org/10.1136/thx.2005.052399
36. Ezzat DA, Morgan DS, Mohamed RA, Mohamed AF. Genetic association of interleukin 18 (-607C/A, rs1946518) single nucleotide polymorphism with asthmatic children, disease severity and total IgE serum level. Cent Eur J Immunol. 2019;44(3):285–91. https://doi.org/10.5114/ceji.2019.89603
37. Shao Y, Zhou Q, Xue J, Shen B, Ji J, Wei Z. Melatonin ameliorates cisplatin-induced asthenozoospermia via reducing oxidative stress. J Mens Health (JOMH). 2022;18(1):016. http://doi.org/10.31083/jomh.2021.127
38. Ma X, Dong J, Wang Y, Zhang P. Protopine regulates oxidative stress, apoptosis and autophagy in H9C2 cardiomyocytes under hypoxic conditions. Signa Vitae. 2024;20(10):113–20. https://doi.org/10.22514/sv.2024.132
39. Zhang L, Zhang Y. Moscatilin inhibits sevoflurane-induced inflammatory and oxidative stress injury in hippocampal neuronal HT22 cells. Signa Vitae. 2024;20(6):67–73. https://doi.org/10.22514/sv.2024.073
40. Pan D, Ye H, Wang J, Mao J. Gentianine facilitates proliferation and inhibits inflammation and oxidative stress in caerulein-triggered acute pancreatitis cell model. Signa Vitae. 2024;20(5):77–84.
41. Zhang C, Xie M, Wang Y, Han T. Tussilagone inhibits inflammation and oxidative stress to alleviate acute pancreatitis. Signa Vitae. 2024;20(1):133–39.
42. Al-Afaleg NO, Al-Senaidy A, El-Ansary A. Oxidative stress and antioxidant status in Saudi asthmatic patients. Clin Biochem. 2011;44(8–9):612–7. https://doi.org/10.1016/j.clinbiochem.2011.01.016
43. Michaeloudes C, Abubakar-Waziri H, Lakhdar R, Raby K, Dixey P, Adcock IM, et al. Molecular mechanisms of oxidative stress in asthma. Mol Aspects Med. 2022;85:101026. https://doi.org/10.1016/j.mam.2021.101026
44. Gwarzo MY, Muhammad SA. Extracellular superoxide dismutase activity and plasma malondialdehyde in human immunodeficiency virus subjects of Kano state as surrogate markers of CD4 status. Int J Biomed Sci. 2010;6(4):294–300. https://doi.org/10.59566/IJBS.2010.6294
45. Jin Y, Huang J, Ma N. In vitro effects of ganoderic acid A on NF-κB-mediated inflammatory response in caerulein-stimulated pancreatic acinar cells. Signa Vitae. 2024;20(6):93–8. https://doi.org/10.22514/sv.2024.075
46. Pan D, Rao X, Gong M, Zhou J. Polygonatum cyrtonema Hua polysaccharides regulates NF-κB and Nrf2 pathways to alleviates oxidative stress and neuroinflammation in cerebral ischemia/reperfusion injury. Signa Vitae. 2024;20(12):108–15. https://doi.org/10.22514/sv.2024.162
47. Mathes E, O'Dea EL, Hoffmann A, Ghosh G. NF-kappa B dictates the degradation pathway of Ikappa B alpha. Embo J. 2008;27(9):1357–67. https://doi.org/10.1038/emboj.2008.73
48. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. Signal Transduct Target Ther. 2017;2:17023. https://doi.org/10.1038/sigtrans.2017.23
49. Trejo Bittar HE, Yousem SA, Wenzel SE. Pathobiology of severe asthma. Annu Rev Pathol. 2015;10:511–45. https://doi.org/10.1146/annurev-pathol-012414-040343
50. Dong J, Liao W, Peh HY, Chan TK, Tan WS, Li L, et al. Ribosomal protein S3 gene silencing protects against experimental allergic asthma. Br J Pharmacol. 2017;174(7):540–52. https://doi.org/10.1111/bph.13717
51. Gu X, Zhang Q, Du Q, Shen H, Zhu Z. Pinocembrin attenuates allergic airway inflammation via inhibition of NF-κB pathway in mice. Int Immunopharmacol. 2017;53:90–5. https://doi.org/10.1016/j.intimp.2017.10.005