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

ORIGINAL ARTICLE

OPEN ACCESS

CXCL16 deficiency alleviates ovalbumin-induced allergic rhinitis in mice

Meihua Kong*, Qian He

Department of ENT, Yueqing People's Hospital, Wenzhou, Zhejiang Province, China

Received 31 March 2025; Accepted 24 May 2025
Available online 1 July 2025

KEYWORDS
allergic rhinitis;
CXCL16;
inflammation;
NF-κB

Abstract
Purpose: Research has indicated that individuals with allergic rhinitis exhibit elevated levels of CXCL16 expression in their serum. This study aims to illustrate the role of CXCL16 and its associated mechanisms in mice suffering from allergic rhinitis.
Methods: An allergic rhinitis model was established by injecting ovalbumin (OVA) into mice, and the expression of CXCL16 mRNA and protein in nasal mucosal tissue was measured. The frequency of nose rubbing and sneezing in each group of mice was recorded. Serum levels of IgE and IgG1 were also assessed. Th2 cell-related factors in the bronchoalveolar lavage fluid (NALF) were analyzed. Histological staining was used to examine pathological changes in the nasal and lung tissues. The expression levels of p-p65, p65, p-IκBα, and IκBα proteins in nasal tissues were evaluated using western blot.
Results: CXCL16 expression was elevated in OVA-induced allergic rhinitis mice. CXCL16 knockout reduced the frequency of nose wiping and sneezing in OVA-induced mice and suppressed the levels of IgE and IgG1. Furthermore, CXCL16 knockout led to a decrease in both the number of inflammatory cells and the levels of inflammatory factors in NALF. Histological staining revealed that CXCL16 knockout alleviated pathological tissue changes and goblet cell hyperplasia. Additionally, CXCL16 knockout suppressed the expression of p-p65/p65 and p-IκBα in nasal tissues, while increasing the expression of IκBα.
Conclusion: CXCL16 deficiency alleviates allergic rhinitis.

***Corresponding author**: Meihua Kong, Department of ENT, Yueqing People's Hospital, No. 338, Qingyuan Road, Chengnan Street, Yueqing City, Zhejiang Province, China. *Email address*: m_hkong0322@163.com

https://doi.org/10.15586/aei.v53i4.1376

Introduction

Allergic rhinitis is a common chronic nasal condition, characterized by persistent mucosal inflammation resulting from the immune system's overreaction to environmental allergens such as dust mites and pollen. The primary symptoms include nasal congestion, rhinorrhea, and paroxysmal sneezing. In clinical practice, immunosuppressants, steroids, and antihistamines are commonly used to manage allergic rhinitis symptoms.[1] However, long-term use of these medications often leads to adverse side effects. Therefore, identifying therapeutic targets is essential for advancing allergic rhinitis research.

When allergens come into contact with the nasal mucosa of hypersensitive individuals, they bind to allergen-specific immunoglobulin E (IgE) on mast cells, triggering early nasal symptoms. Mast cells release histamine and tumor necrosis factor-alpha (TNF-α), which promote the expression of adhesion molecules on endothelial cells and enhance the infiltration of inflammatory cells.[2] In allergic rhinitis (AR), nuclear factor kappa B (NF-κB) is activated, leading to an inflammatory response.[3]

CXCL16 is a chemokine belonging to the CXC subfamily.[4] It is expressed in tissues such as the liver, spleen, kidney, and lung, especially in macrophages, dendritic cells, and endothelial cells.[5] CXCL16 is regulated by inflammatory factors and pathogen-associated molecular patterns (PAMPs). In mice, acetaminophen-induced hepatotoxicity is lessened by CXCL16 deficiency by lowering inflammation and oxidative stress in the liver.[6] CXCL16 reduces the expression of neuroinflammatory factors, enhances anti-inflammatory factors, and alleviates cerebral hemorrhage in mice.[7] CXCL16 impairs myocardial function by activating NF-κB and p38 MAPK signaling pathways.[8] Research has indicated that the serum of AR patients has elevated levels of CXCL16 expression.[9]

There are relatively few studies on the role and related mechanisms of CXCL16 in allergic rhinitis. According to this study, CXCL16 is significantly expressed in allergic rhinitis, and knocking down CXCL16 can alleviate the symptoms in allergic rhinitis mice, which may be related to CXCL16 regulating the NF-κB pathway.

Methods

Biological analysis

The GSE52804 expression profile was selected from the GEO dataset and divided into a control group and an OVA pathogenic group to analyze the differential gene expression in the nasal mucosal tissues of the two groups.

Animals

Male, 6–8-week-old C57BL/6J wild-type (WT) and CXCL16 KO mice with a C57BL/6J genetic background were obtained from Cyagen (Suzhou, China) (n=8). On days 0, 7, and 14, the mouse model was induced with ovalbumin (OVA) via intraperitoneal injection of 100 μL saline containing 50 μg OVA and 2 mg $Al(OH)_3$. From day 21 to 28, 20 μL of saline containing 400 μg OVA was administered intranasally. Following the final intranasal administration, the number of sneezes and nose wipes was recorded over a fifteen-minute period. All animal experiments were conducted in accordance with international and national standards for animal care and ethics, with approval from the Animal Ethics Committee.

Detection of specific immunoglobulins in serum

Blood samples were collected from the orbital sinus, and the blood was allowed to stand at 4°C for 30 minutes. The samples were then centrifuged at 3000 rpm for 10 minutes, and the supernatant was collected as serum. Anti-IgG1 or IgE antibody was diluted and added to a 96-well plate, followed by incubation at 37°C for 2 hours. The plate was washed three times with washing buffer. Blocking solution was then added and incubated at 37°C for 1 hour. Diluted serum samples were added and incubated at 37°C for another hour. HRP-labeled secondary antibody was added and incubated at 37°C for 1 hour. After washing the plate, TMB substrate was added and allowed to react at room temperature in the dark for 10–15 minutes. Stop solution was then added, and absorbance was read at 450 nm using an enzyme reader. The concentrations of IgG1 or IgE were calculated based on the standard curve formula.

Cell counting

The nasal lavage fluid (NALF) of mice was collected and centrifuged to remove the supernatant. The cell pellet was resuspended in PBS, and the total number of cells was counted using the trypan blue method. Diff-Quik staining was used to identify and count different cell types.

Detection of IL-4, IL-5, IL-13, and IL-6 in NALF

Dilute the capture antibody to the concentration specified in the instructions using coating buffer, add 100 μL to each well of a 96-well plate, and incubate at 37°C for 2 hours. Discard the coating solution, fill each well with 200 μL of blocking solution, and incubate at 37°C for 1 hour. Add the standard and the supernatant of the nasal lavage fluid, and incubate at 37°C for 2 hours. Add the biotinylated detection antibody and enzyme conjugate, then add the colorimetric solution followed by the stop solution. Finally, measure the OD value using a microplate reader, and calculate the concentration corresponding to the sample OD value based on the standard curve equation. IL-6 (MlBio, MI098430), IL-5 (MlBio, MI063157), IL-4 (MlBio, MI064310), IL-13 (MlBio, MI106729).

H&E and PAS staining

The nasal and lung tissue samples were embedded in paraffin and sectioned at a thickness of 4 μm. The sections were stained according to the H&E staining protocol. In addition, goblet cells, which are the primary cell

type responsible for mucus secretion, are closely associated with respiratory inflammation, infection, and allergic diseases. Therefore, the sections were also stained using the periodic acid Schiff (PAS) staining protocol to evaluate goblet cell proliferation.

PCR

Frozen tissue samples were ground into powder in liquid nitrogen and then homogenized using RNA extraction reagent (TRIzol). RNA samples were treated with DNase I to eliminate potential genomic DNA contamination. The RNA was reverse transcribed into cDNA using reverse transcriptase and random primers. PCR amplification was performed according to the cycling conditions required for CXCL16 primers and the specifications of the PCR instrument. Quantification was carried out using the 2^-ΔΔCt method. Forward primer (5'-TCGCTGGAAGTTGTTCTTGTGA-3'); Reverse primer (5'-GACCAGTTCCACACTCTTTGCG-3').

Western-blot

Nasal mucosa tissue was ground into a powder using liquid nitrogen, followed by homogenization with RIPA lysis buffer, centrifugation, and collection of the supernatant for protein concentration analysis using a BCA kit. The samples were loaded into electrophoresis-ready gel wells, and the separated protein bands were transferred to a PVDF membrane and blocked with 5% skim milk powder. The membrane was incubated with the primary antibody overnight at 4°C, followed by incubation with the secondary antibody at room temperature. Protein bands were visualized using an ECL luminescence kit and a gel imaging device. CXCL16 (GeneTex, GTX116706, 1:1000). p-p65 (Abcam, ab76302, 1:1000). P65 (Abcam, ab32536, 1:1000). IκBα (Affinity, AF5002, 1:1000). p - IκBα (Affinity, AF2002, 1:1000). ß-actin (Affinity, AF7018, 1:1000).

Statistical analysis

The data were analyzed using SPSS software. Comparisons between two groups were performed using the Student's t-test, while comparisons among multiple groups were conducted using one-way analysis of variance. Tukey's HSD method was used for post hoc testing following ANOVA.

Results

CXCL16 is highly expressed in the nasal mucosa of AR mice

The analysis of the GSE52804 expression profile revealed that CXCL16 expression was elevated in the nasal mucosa of allergic rhinitis (AR) mice (Figure 1A). The expression of CXCL16 in the nasal mucosa tissues of wild-type mice and OVA-induced allergic rhinitis mice was assessed through PCR and western blot. The nasal mucosa of allergic rhinitis mice exhibited higher mRNA levels of CXCL16 (Figure 1B) and protein expression (Figure 1C), as indicated by the results.

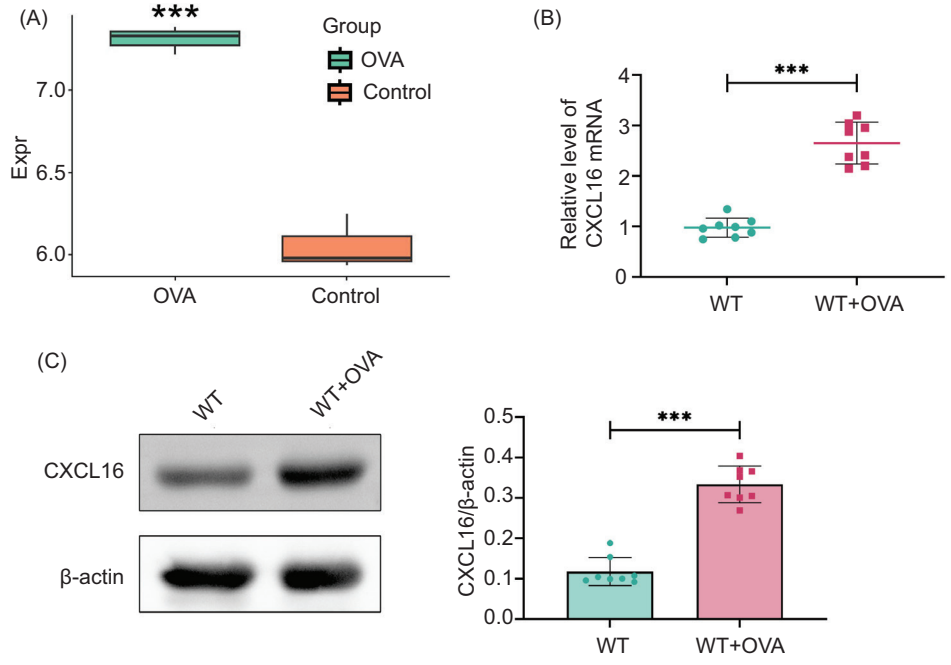

Figure 1 CXCL16 is highly expressed in the nasal mucosa of AR mice. (A) Expression of CXCL16 in the GSE52804 expression profile in the nasal mucosal tissues of mice with OVA-induced allergic rhinitis. (B) Detection of CXCL16 mRNA expression in the nasal mucosa of mice with OVA-induced allergic rhinitis by PCR. (C) Detection of CXCL16 protein expression in the nasal mucosa of mice with OVA-induced allergic rhinitis by western blot vs .control, ***P <0.001. vs .WT, ***P <0.001.

CXCL16 deficiency improves symptoms and OVA-specific immunoglobulins in serum of AR mice

OVA-induced wild-type (WT) mice showed increased frequencies of nose wiping and sneezing, whereas CXCL16 knockout (KO) mice exhibited reduced frequencies of rubbing and sneezing induced by OVA (Figure 2A). Additionally, mouse serum levels of IgE and IgG increased as a result of OVA exposure, while CXCL16 KO mice demonstrated a reduction in the levels of both IgE and IgG (Figure 2B).

CXCL16 deficiency reduces inflammatory cell infiltration in NALF

The total number of cells, including epithelial cells, eosinophils, macrophages, neutrophils, and lymphocytes, in the NALF of OVA-induced wild-type (WT) mice increased, whereas CXCL16 knockout (KO) mice exhibited a reduction in the number of these cells (Figure 3A). Additionally, the levels of IL-4, IL-5, IL-13, and IL-6 in the NALF of mice were measured using kits. The results showed that OVA elevated the levels of the aforementioned inflammatory factors, while CXCL16 KO reduced the levels of these inflammatory factors (Figure 3B).

CXCL16 deficiency alleviates pathological changes in nasal mucosal tissues of AR mice

Mice in the OVA group exhibited significantly thicker mucosa and a higher aggregation of inflammatory cells, as shown by H&E staining of their lung and nasal tissues (Figure 4A). PAS staining revealed that the goblet cells in the OVA group were hyperplastic (Figure 4B). The pathological alterations induced by OVA were alleviated in CXCL16 knockout (KO) mice.

CXCL16 deficiency inhibits the NF-κB pathway

Western blotting was used to assess the expression of p-p65, p65, p-IκBα, and IκBα in nasal mucosal tissue. The results showed that OVA injection increased the levels of

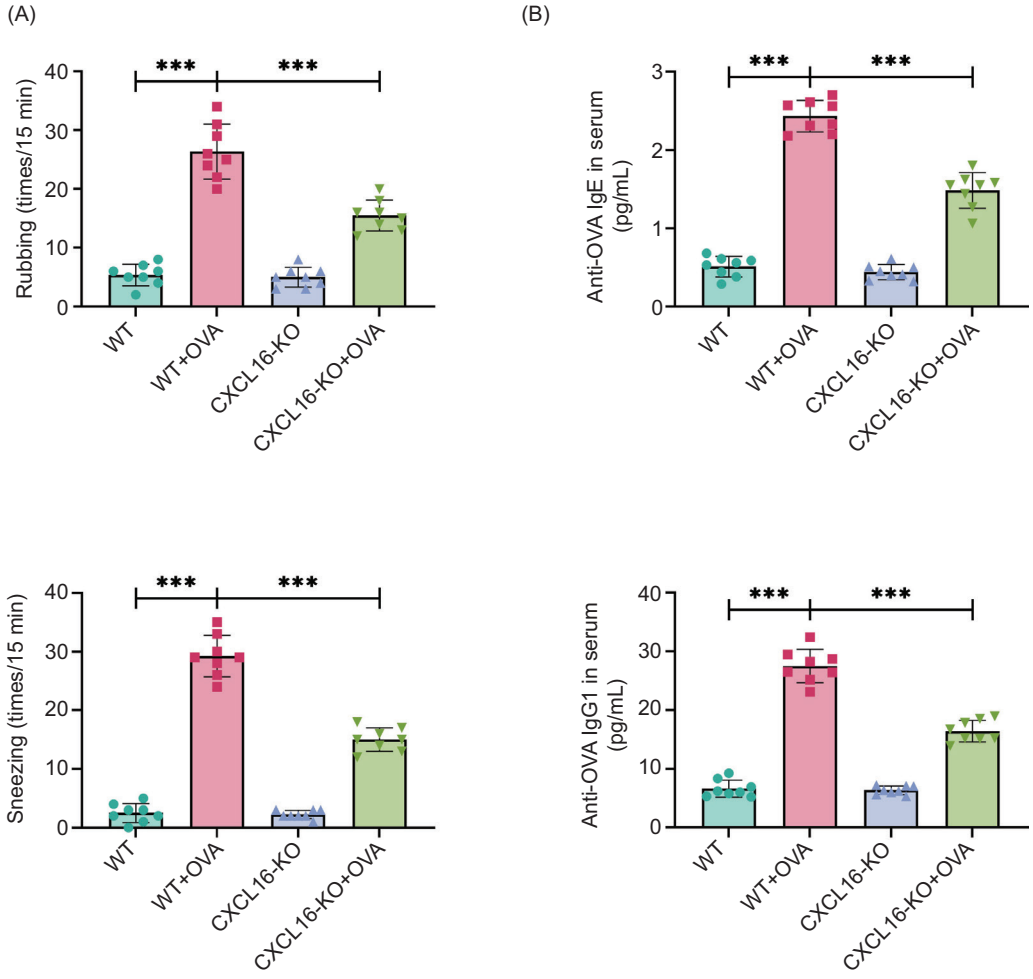

Figure 2 CXCL16 deficiency improves symptoms and OVA-specific immunoglobulins in serum of AR mice. (A) Observe and record the number of times the mouse wipes its nose and sneezes within a 15-minute period. (B) Kit for detecting the levels of IgE and IgG1 in mouse serum. ***P <0.001.

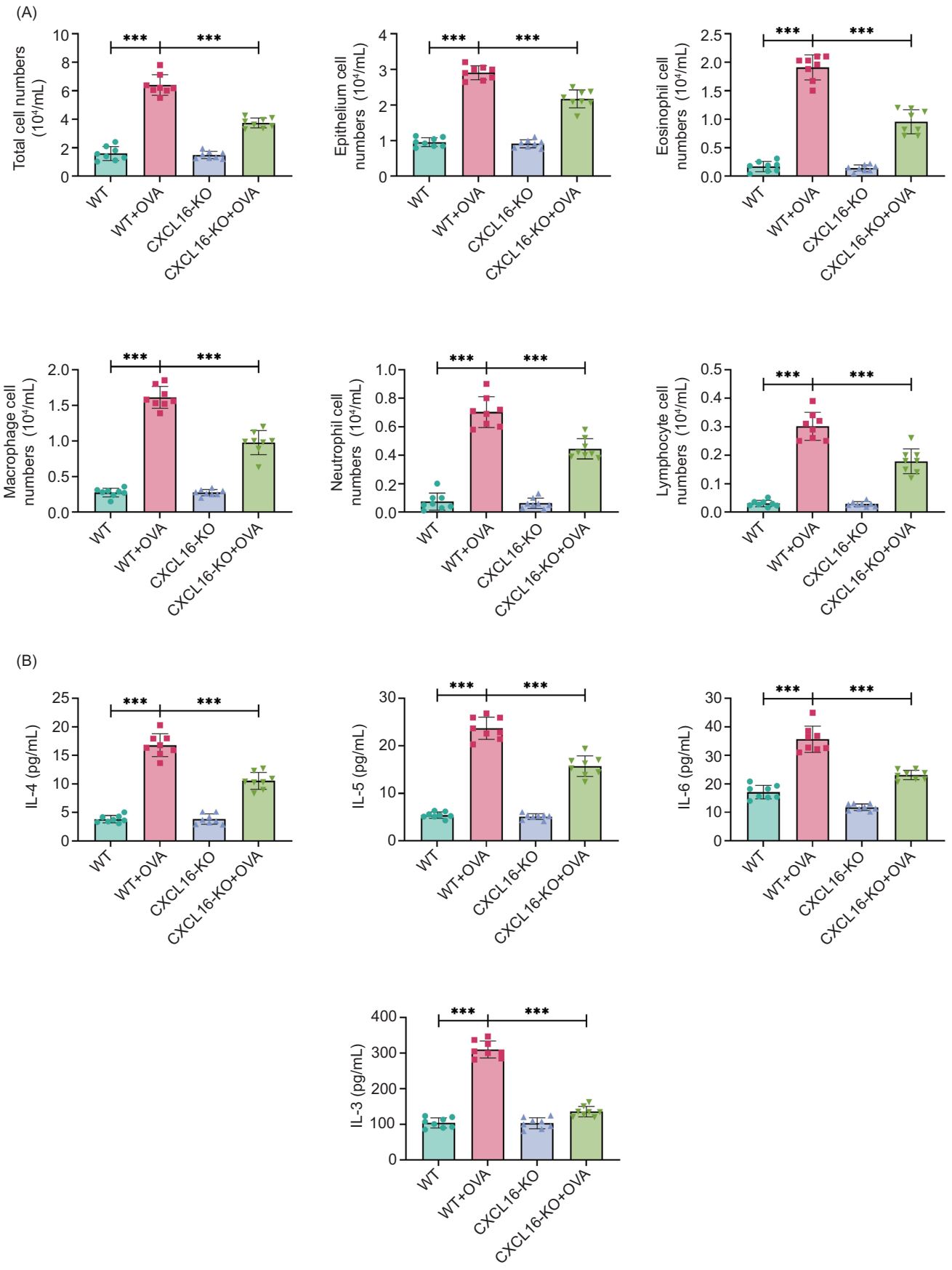

Figure 3 CXCL16 deficiency reduces inflammatory cell infiltration in NALF. (A) Count the total number of cells, epithelial cells, eosinophils, macrophages, neutrophils, and lymphocytes in the NALF using a cell counter. (B) Kit for detecting IL-4, IL-5, IL-13, and IL-6 in mouse NALF. ***P <0.001.

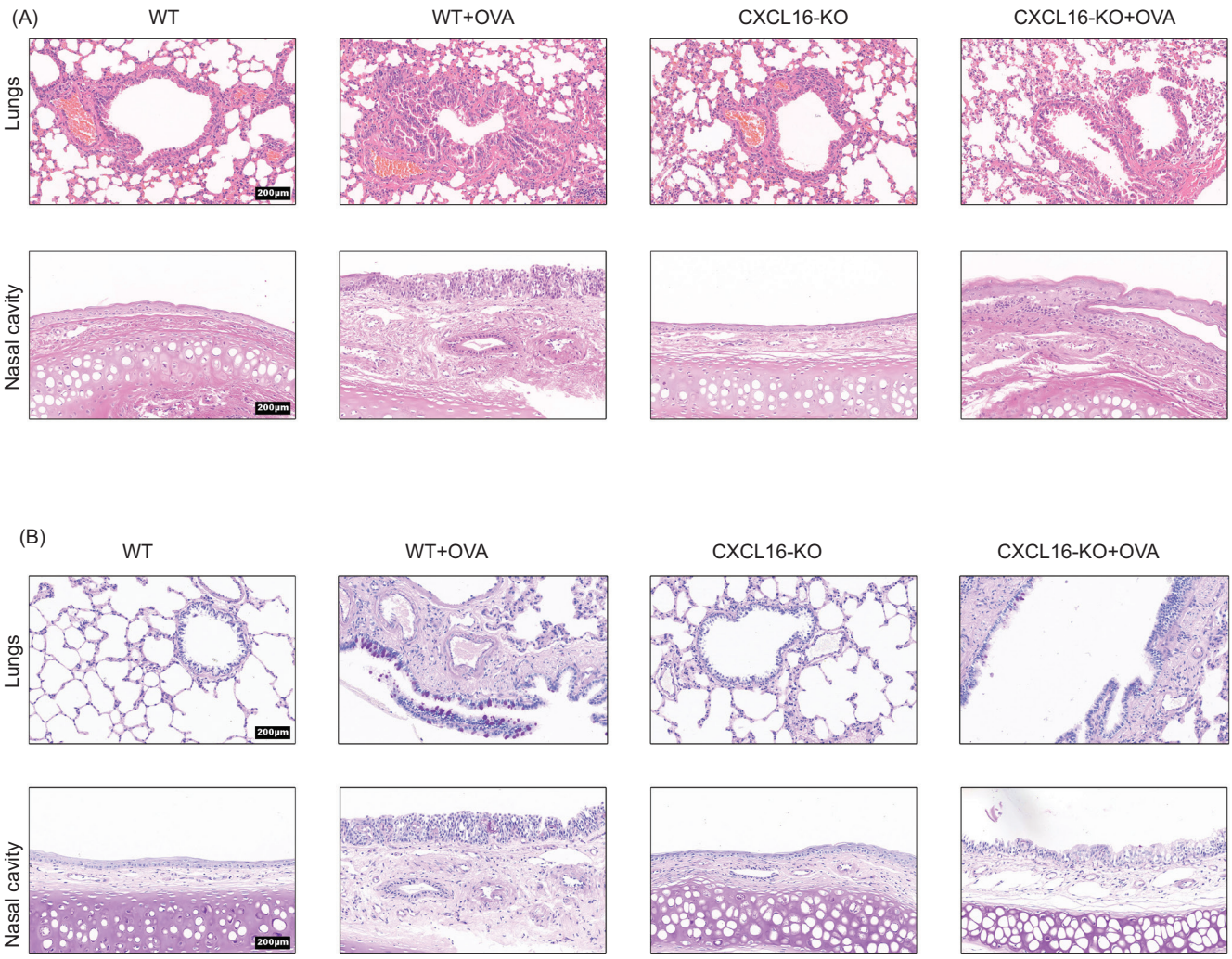

Figure 4 CXCL16 deficiency alleviates pathological changes in nasal mucosal tissues of AR mice. (A) H&E staining to examine the pathological changes in the lung and nasal tissues of mice. (B) PAS staining to assess goblet cell proliferation in mouse lung and nasal tissues.

p-p65/p65 and p-IκBα, while decreasing the expression of IκBα. In contrast, CXCL16 knockout (KO) reduced the expression of p-p65/p65 and p-IκBα in nasal mucosal tissue induced by OVA and increased the expression of IκBα (Figure 5). These findings suggest that CXCL16 KO suppresses the NF-κB pathway.

Discussion

After exposure to allergens, IgE primarily mediates the chronic immunological inflammatory condition known as allergic rhinitis. By facilitating the activation of mast cells and basophils, IgE induces the production of inflammatory components, including histamine, leukotrienes, and Th2 cytokines, which lead to allergy symptoms such as sneezing, itching, rubbing, and increased secretion in the nasal cavity.[10] Inflammation of the upper respiratory tract can spread to the lower respiratory tract, potentially triggering asthma.[11] CXCL16's ability to bind and internalize modified forms of oxidized low-density lipoprotein, acting as a scavenger receptor, is crucial for clearing apoptotic cells and facilitating the uptake of various pathogens. Under proteolytic cleavage by the metalloproteinase ADAM10, the chemokine domain in its extracellular region generates the soluble chemokine sCXCL16, which exerts chemokine activity.[12] Elevated levels of CXCL16 expression have been observed in both the lung tissues of asthma mice and patients. In the case of Aspergillus-induced asthma, treatment with CXCL16 deletion resulted in reduced airway inflammation, mucus production, and airway hyperresponsiveness.[13] Since both asthma and allergic rhinitis are primarily triggered by allergens and involve airway inflammation, it is hypothesized that CXCL16 may play a role in allergic rhinitis. This study demonstrated that the nasal mucosa of mice with OVA-induced allergic rhinitis exhibited significant CXCL16 expression. CXCL16 knockout mice showed reduced rubbing and sneezing, along with suppressed levels of IgE and IgG.

IL-4, IL-5, and IL-13 are Th2 cytokines. IL-4 promotes B cell production of IgE, facilitates Th2 cell differentiation, increases IgE levels, and triggers allergic reactions. IL-5

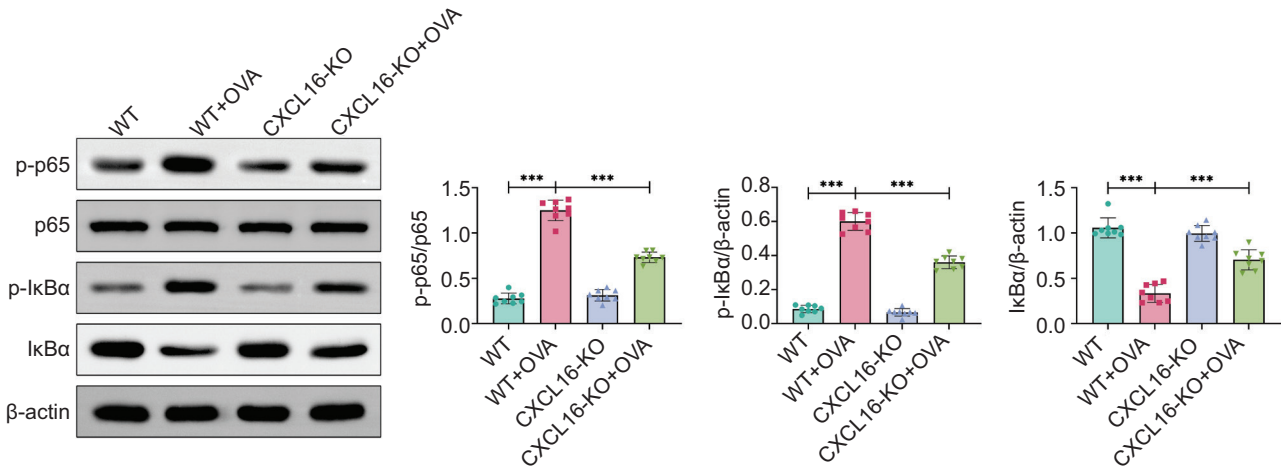

Figure 5 CXCL16 deficiency inhibits the NF-κB pathway. Protein expressions of p-p65, p65, p-IκBα, and IκBα in mouse nasal tissues were assessed by western blot. ***P <0.001.

activates eosinophils, leading to nasal mucosal inflammation and tissue damage. IL-13 promotes mucus secretion and airway hyperresponsiveness, participates in IgE production, and contributes to nasal congestion and mucus production.[14] IL-6 exacerbates the inflammatory response and contributes to tissue damage. In this study, OVA exposure led to increased levels of Th2 cytokines and IL-6 in mice, while CXCL16 knockout reduced the levels of these cytokines and IL-6.

The NF-κB pathway plays a crucial role in the inflammatory response of allergic rhinitis. Allergens, cytokines (such as TNF-α and IL-1β), and pathogen-associated molecular patterns (PAMPs) trigger NF-κB activation. The IκB kinase (IKK) complex phosphorylates the IκB protein, leading to its degradation, which releases NF-κB dimers. These dimers then enter the cell nucleus, bind to DNA, and initiate gene transcription. NF-κB promotes the expression of cytokines such as IL-1β, IL-6, and TNF-α, thereby exacerbating inflammation. It also enhances the production of Th2 cytokines, such as IL-4, IL-5, and IL-13, which drive Th2-type immune responses. NF-κB plays a role in the activation and survival of eosinophils, further aggravating inflammation and tissue damage. Additionally, NF-κB indirectly promotes B cells to produce IgE by upregulating the expression of IL-4 and IL-13.[15] In this study, OVA exposure led to increased expression of p-p65/p65 and p-IκBα, along with decreased expression of IκBα in the nasal mucosal tissue of mice, while CXCL16 knockout inhibited the NF-κB pathway.

The study concluded that CXCL16 was highly expressed in the nasal mucosa of mice with OVA-induced allergic rhinitis. CXCL16 knockout reduced allergic rhinitis symptoms and inhibited Th2 cytokines, which may be linked to the inhibition of the NF-κB pathway by CXCL16 knockout.

Ethics Approval

Ethical approval was obtained from the Ethics Committee of Ethics Committee of Yueqing Hospital of Wenzhou Medical University (Approval no. YQYY202400223).

Data Availability

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Author Contributions

MK and QH designed the study and carried them out, supervised the data collection, analyzed the data, interpreted the data, prepare the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

Conflict of Interests

The authors declare no conflict of interest.

Funding

No funding was used in this study.

References

1. Zhou X, Song X, Shu T, Zhang S, Zhang Z, Hu C, et al. Prevention and alleviation of allergic rhinitis by oral administration of Lacticaseibacillus paracasei GOLDGUT-Lpc969. Front Immunol. 2024;15:1444778. https://doi.org/10.3389/fimmu.2024.1444778
2. Yu ZN, Fan YJ, Nguyen TV, Piao CH, Lee BH, Lee SY, et al. Undaria pinnatifida ameliorates nasal inflammation by inhibiting eosinophil and mast cell activation and modulating the NF-κB/MAPKs signaling pathway. Immun Inflamm Dis. 2024;12(3):e1215. https://doi.org/10.1002/iid3.1215
3. Piao CH, Fan YJ, Nguyen TV, Song CH, Chai OH. Mangiferin Alleviates Ovalbumin-Induced Allergic Rhinitis via Nrf2/HO-1/NF-κB Signaling Pathways. Int J Mol Sci. 2020;21(10). https://doi.org/10.3390/ijms21103415

4. Zhang W, Hua T, Li J, Zheng L, Wang Y, Xu M, et al. CXCL16 is activated by p-JNK and is involved in H(2)O(2)-induced HK-2 cell injury via p-ERK signaling. Am J Transl Res. 2018;10(11):3723-32.
5. Lepore F, D'Alessandro G, Antonangeli F, Santoro A, Esposito V, Limatola C, et al. CXCL16/CXCR6 Axis Drives Microglia/Macrophages Phenotype in Physiological Conditions and Plays a Crucial Role in Glioma. Front Immunol. 2018;9:2750. https://doi.org/10.3389/fimmu.2018.02750
6. Wang H, Shao Y, Zhang S, Xie A, Ye Y, Shi L, et al. CXCL16 deficiency attenuates acetaminophen-induced hepatotoxicity through decreasing hepatic oxidative stress and inflammation in mice. Acta Biochim Biophys Sin. 2017;49(6):541-9. https://doi.org/10.1093/abbs/gmx040
7. Dingyi L, Libin H, Jifeng P, Ding Z, Yulong L, Zhangyi W, et al. Silencing CXCL16 alleviate neuroinflammation and M1 microglial polarization in mouse brain hemorrhage model and BV2 cell model through PI3K/AKT pathway. Exp Brain Res. 2024;242(8):1917-32. https://doi.org/10.1007/s00221-024-06875-y
8. Zhang J, Hao W, Zhang J, Li T, Ma Y, Wang Y, et al. CXCL16 Promotes Ly6Chigh Monocyte Infiltration and Impairs Heart Function after Acute Myocardial Infarction. J Immunol. 2023;210(6):820-31. https://doi.org/10.4049/jimmunol.2200249
9. Zhang Y, Li J, Si Y. Changes of serum CXCL16 and ANCA levels in allergic rhinitis and their clinical significance. Int J Lab Med. 2024;12:330-4.
10. Chen F, He D, Yan B. Apigenin Attenuates Allergic Responses of Ovalbumin-Induced Allergic Rhinitis Through Modulation of Th1/Th2 Responses in Experimental Mice. Dose Response. 2020;18(1):1559325820904799. https://doi.org/10.1177/1559325820904799
11. Chen Q, Shao L, Li Y, Dai M, Liu H, Xiang N, et al. Tanshinone IIA alleviates ovalbumin-induced allergic rhinitis symptoms by inhibiting Th2 cytokine production and mast cell histamine release in mice. Pharm Biol. 2022;60(1):326-33. https://doi.org/10.1080/13880209.2022.2034894
12. Xia Y, Entman ML, Wang Y. Critical role of CXCL16 in hypertensive kidney injury and fibrosis. Hypertension. 2013;62(6):1129-37. https://doi.org/10.1161/HYPERTENSIONAHA.113.01837
13. Liu TT, Zhang Z, Deng J, Shi CY, Zheng S, Jia LX, et al. CXCL16 knockout inhibit asthma airway inflammation by suppressing H2-DM molecular mediated antigen presentation. Cell Death Discov. 2025;11(1):90. https://doi.org/10.1038/s41420-025-02371-6
14. Dong F, Tan J, Zheng Y. Chlorogenic Acid Alleviates Allergic Inflammatory Responses Through Regulating Th1/Th2 Balance in Ovalbumin-Induced Allergic Rhinitis Mice. Med Sci Monit. 2020;26:e923358. https://doi.org/10.12659/MSM.923358
15. Jin J, Fan YJ, Nguyen TV, Yu ZN, Song CH, Lee SY, et al. Fallopia japonica Root Extract Ameliorates Ovalbumin-Induced Airway Inflammation in a CARAS Mouse Model by Modulating the IL-33/TSLP/NF-κB Signaling Pathway. Int J Mol Sci. 2023;24(15). https://doi.org/10.3390/ijms241512514