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ORIGINAL RESEARCH

Role of ILC2s as Potential Effector Cells of IL25-Mediated Type 2 Inflammation in Chronic Rhinosinusitis with Nasal Polyps in China

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Introduction: Chronic rhinosinusitis with nasal polyps (CRSwNP) is characterized by Th2-type inflammation and is associated with dysregulated interleukin-25 (IL-25) expression. Type II innate lymphoid cells (ILC2s), as potential effector cells of IL-25, may contribute to the pathogenesis of CRSwNP. However, their specific role in nasal polyp (NP) tissues, particularly in Chinese patients, remains insufficiently understood.

Methods: Nasal polyp (NP) tissue and turbinate mucosa (TM) were collected from 37 Chinese CRSwNP patients undergoing surgery. TM samples from 7 patients with pituitary tumors were used as controls. IL-25 expression, Th2 cytokines, phosphorylated STAT3 (p-STAT3), and ILC2 levels were assessed via immunohistochemistry, flow cytometry, and ELISA. Isolated ILC2s from NP tissues were stimulated with IL-25, with or without limonin treatment, to evaluate downstream cytokine production and STAT3 activation.

Results: Compared to control TM, both NPs and TM from CRSwNP patients showed elevated IL-25 expression. NP tissues exhibited increased p-STAT3 levels and overexpression of Th2 cytokines. ILC2s were significantly more abundant in NPs and TM of CRSwNP patients. Upon IL-25 stimulation, NP-derived ILC2s produced higher levels of IL-5 and IL-13, accompanied by enhanced STAT3 phosphorylation. Limonin treatment significantly reduced both STAT3 activation and Th2 cytokine production in IL-25-stimulated NP tissues.

Conclusion: ILC2s function as key effector cells of IL-25 in CRSwNP, promoting type-2 inflammation via the STAT3 signaling pathway. Limonin attenuates this response and may serve as a promising therapeutic agent for CRSwNP by targeting IL-25/STAT3-driven ILC2 activation.

Keywords: chronic sinusitis, nasal polyps, turbinate mucosa, interleukin-25, IL-25, group 2 innate lymphoid cells, ILC2s, STAT3 signaling pathway, limonin

Introduction

Chronic rhinosinusitis (CRS) is a common disease marked by elevated levels of T helper (Th) cytokines, chronic rhinosinusitis with nasal polyps (CRSwNP) is closely associated with type 2 inflammation showing high concentrations of Th2 cytokines such as IL-5 and IL-13.[1–3] Recent research suggests that these cytokines are produced not only by T cells but also by other immune cells, such as mast cells, basophils, and group 2 innate lymphoid cells (ILC2s).[4–6] As a result, Th2 cytokines are now broadly referred to as type 2 cytokines. In Western countries, CRSwNP is predominantly associated with type 2 inflammation, characterized by pronounced eosinophilia and elevated levels of type 2 cytokines including IL-5 and IL-13.[7] However, while less frequent, the occurrence of this type 2 inflammation in NPs is increasing among Asian patients as well.[8,9]

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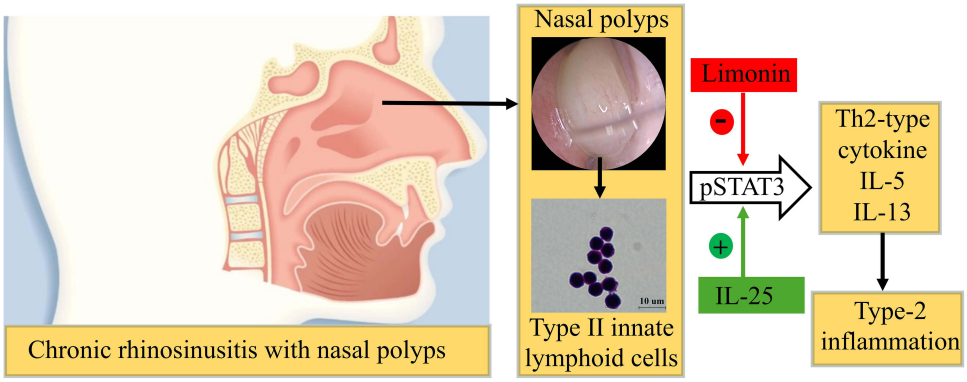
Graphical Abstract



Interleukin (IL)-25, which is a member of the IL-17 cytokine family and known as IL-17E, plays a key role in inducing and regulating type 2 inflammation.[10–13] IL-25 is mainly produced by epithelial cells, Th2 cells, mast cells, eosinophils, macrophages, dendritic cells, and basophils.[10,14–18] In mouse model, IL-25 has been shown to play an important role in the pathogenesis of CRSwNP.[19] Targeting IL-25 neutralizing antibodies can reduce the number of polyps and inflammatory status in murine NPs.[19] However, the role of IL-25 on NPs development in human is still controversial, and the expression of IL-25 in NPs is likely related to ethnicity. Research in the United States using a larger cohort found that IL-25 levels were nearly undetectable in NPs.[20] In contrast, studies from Asian countries have reported elevated IL-25 levels in NPs, suggesting that IL-25 may be involved in the pathogenesis of eosinophilic NPs specifically in Asian populations.[20] However, a recent study in the US found IL-25 in the NP is primarily produced by solitary chemosensory cells that is a subset of epithelial cells.[21] This indicates that the quantity of these cells in NPs may influence IL-25 detection and suggests that IL-25 could play a role in NP pathogenesis even in Western populations. Further research is needed to clarify the role of IL-25 and its effector cells in the NPs from CRSwNP patients.

ILC2s function as an important role in type 2 inflammation and can be activated to produce a large number of Th2 cytokines IL-4, IL-5 and IL-13 under the influence of upper dermal cytokines IL-25, IL-33 and thymic stromal lymphopoietin (TSLP), which are involved in the process of immune reaction.[22–27] Research has shown that ILC2 were dominant and significantly elevated in NPs compared to PBMC, tonsil, and normal sinus tissue in CRSwNP patients.[28] Our previous research has shown that IL-25 is able to regulate pathogenesis of asthma and allergic rhinitis primarily through ILC2s sorted from peripheral blood mononuclear cells.[29] However, under IL-25 stimulation, the role of ILC2s in NPs from Chinese CRSwNP patients remains uninvestigated.

Thus, this study aimed to examine the impact of IL-25 on the ILC2s isolated from NPs of Chinese CRSwNP patients in response to IL-25 stimulation. In vitro studies were used to investigate the potential signaling pathway involved in the effects of IL-25 on ILC2s in CRSwNP diseases.

Methods

Study Subjects

The study was approved by the Ethics Committee of The First Affiliated Hospital of Zhengzhou University (2024-KY -0405-002) in accordance with the Declaration of Helsinki. Written informed consent was obtained from 44 subjects (ages 16–65), including 37 patients with CRSwNP and 7 control patients with pituitary tumors but without CRS. All patients underwent endoscopic sinus surgery were diagnosed with CRSwNP according to EPOS 2020 guidelines and were refractory to standardized medical treatment.

Inclusion Criteria for CRSwNP Patients

1. All participants met the diagnostic criteria outlined in the 2012 European Position Paper on Sinusitis and Nasal Polyps (EPOS) and had a disease duration of more than 12 weeks with nasal polyps.
2. Primary symptoms: Nasal congestion and/or sticky or purulent nasal discharge.
3. Secondary symptoms: Facial swelling and a decreased or lost sense of smell. Diagnosis required at least two of the symptoms listed above.
4. Nasal endoscopy findings: Presence of viscous or mucopurulent secretions in the middle nasal passage and olfactory fissure, along with nasal mucosa congestion, edema, or visible polyps.
5. Imaging findings: CT scans showing inflammatory lesions of the ostiomeatal complex and/or sinus mucosa.

Exclusion Criteria

1. Use of nasal spray hormones or oral hormone therapy within the past month.
2. Symptoms of acute upper respiratory tract infection within the past month.
3. Pregnancy.
4. Serious comorbid organ or systemic diseases (eg, heart, liver, kidney), immunodeficiency (eg, AIDS), abnormal coagulation function, or neoplastic diseases.

Collection and Treatment of Specimens

Samples were collected from the NPs and TM of CRSwNP patients, as well as from the TM of non-CRS pituitary tumor patients. Immunohistochemical methods were used to detect IL-25 levels in these tissues. The level of ILC2s was measured using flow cytometry after single-cell staining. NPs from CRSwNP patients were divided into two groups: one cultured with DMEM (Gibco, USA) alone and the other stimulated with DMEM plus IL-25 (Human IL-17E (IL-25) Recombinant Protein, PeproTech®) for 12 hours at 10 ng/mL. The levels of IL-5 and IL-13 in the supernatant were measured using the ELISA kits (Abcam, USA).

Protein was extracted from the NPs to analyze the phosphorylation levels of STAT3, using Western blotting.

Western Blot

Western blot experiments were carried out by following the well-established protocols with modifications.[19,29] Proteins were extracted from nanoparticles (NPs) subjected to various experimental conditions. An equal amount of protein (15 μg per lane) was loaded onto SDS-PAGE gels for electrophoretic separation, followed by transfer onto polyvinylidene difluoride (PVDF) membranes (Roche Diagnostics, Indianapolis, IN). Membranes were blocked using 10% nonfat dry milk, rinsed, and then incubated with primary antibodies against phosphorylated STAT3 (Tyr705) (D3A7) XP rabbit monoclonal antibody (1:500 dilution) and GAPDH mouse monoclonal antibody (1:10000 dilution). Following additional washes, membranes were treated with horseradish peroxidase (HRP)-conjugated secondary antibodies targeting rabbit or mouse IgG. Protein bands were visualized using Enhanced Chemiluminescence Plus detection reagent (Millipore Corporation, Billerica, MA). All antibodies used were sourced from Cell Signaling Technology (Danvers, MA).

Enzyme-Linked Immunosorbent Assay (ELISA)

The levels of human IL-5, IL-13 in the supernatants of extraction of nasal polys and ILC2s cell-culture were examined using commercially available ELISA kits (RayBiotech Inc, Norcross, GA for IL-5 and IL-13 in accordance with the manufacturer's instructions. The detection limits were 2.74 pg/mL for IL-5, 0.15 pg/mL for IL-13. All values below the detection limits were set at 0.

ILC2s Isolation and Cell Sorting from Human NP Tissue

ILC2s were sorted from patients' NPs with CRSwNP as previously described.[30] Tissue samples were fragmented and incubated with 30 μg/mL DNase I and 1 mg/mL type I collagenase containing media at 4 °C overnight. Following this, tissues were minced using dissociator, and the cells were filtered through 70 -μm nylon mesh (BD Biosciences, San Jose, CA). Cells were then treated with red blood cell lysis solution (Miltenyi Biotec) before counting and staining for cell

sorting. After the isolation, cells were first treated with Aqua LIVE/DEAD fixable dead cell staining reagent (Invitrogen, Carlsbad, CA) at room temperature in the dark. Cells were then blocked by Fc Block reagent and incubated with Human Hematopoietic Lineage FITC Cocktail (eBioscience, USA), Anti-Human Fc epsilon Receptor I alpha (FceR1) FITC (eBioscience, USA), PE-Cy7 Mouse Anti-Human CD127 (BD, USA) and PE Rat Anti-Human CD294 (CRTH2) (BD, USA) at 4 °C in the dark. We sorted ILC2s ($Lin^{-}FceR1^{-}CRTH2^{+}CD127^{+}$ lymphocytes) with a BD FACSAria SORP cell sorter.

Cell Culture

Sorted NPs ILC2s, were suspended in the RPMI medium supplemented with 25 IU/mL IL-2 (Prometheus, San Diego, CA), 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin, as previously described.[30] ILC2s were stimulated with 100 ng/mL IL-25 (R&D Systems, Minneapolis, MN), with addition of limonin (stored in DMSO at 5 mM) at 100 μM for 30 minutes. After the centrifuge, the expression of IL-5 and IL-13 was quantified by ELISA kits (Abcam, USA). After the protein extraction, the phosphorylation of STAT3 was quantified by Western blot.

Immunohistochemistry

IHC of NPs and TM tissues was performed as previously reported with modifications.[19] Immunohistochemical (IHC) staining was performed using the Polink-2 HRP Plus Broad DAB Detection System (Golden Bridge International Labs, Bothell, WA). After deparaffinization, the tissue sections were treated with 3% hydrogen peroxide to block endogenous peroxidase activity and subjected to heat-induced epitope retrieval in 10 mmol/L citrate buffer (pH 6.0) using a microwave. The sections were then incubated at room temperature for 60 minutes with a primary antibody, rabbit anti-human IL-25 (1:500, Abcam). Following this, incubation was carried out using a broad antibody enhancer, polymer-horseradish peroxidase (HRP), and staining with the DAB Detection System. Hematoxylin was used as a counterstain. Sequential IHC was performed using polymer-HRP and alkaline phosphatase kits to detect primary antibodies in human tissue, with Permanent Red and Emerald staining (Polink DS-MR-Hu C2 Kit, Golden Bridge International Labs) used to identify IL-25. Polymer mixtures were created by combining alkaline phosphatase polymer anti-mouse IgG and polymer-HRP anti-rabbit IgG in a 1:1 ratio and used as negative control.

Flow-Cytometric Analysis

Flow-cytometry was utilized to count ILC2s as previously described.[30] Cells were first treated with Aqua dead cell staining reagent as a live/dead discriminator. Cells were then incubated with an Fc Block reagent (Miltenyi Biotec) for 10 min at 4 °C in the dark. Cells were stained with the following antibodies: Human Hematopoietic Lineage FITC Cocktail (eBioscience, USA), Anti-Human Fc epsilon Receptor I alpha (FceR1) FITC (eBioscience, USA), PE-Cy7 Mouse Anti-Human CD127 (BD, USA) and PE Rat Anti-Human CD294 (CRTH2) (BD, USA). Cells were stained for 30 min at 4 °C in the dark and washed with MACS buffer (Miltenyi Biotech). After washing, cells were fixed with a BD Cytofix/Cytoperm Kit (BD Biosciences), resuspended in MACS buffer, and stored at 4 °C in the dark before analysis on a CytoFLEX flow cytometer (Beckman Coulter, Indianapolis, IN). All analysis was performed with FlowJo software, version 10.1 (TreeStar, Ashland, OR), and the experimental method was established and verified with the proper single-stained control beads (BD Biosciences and eBiosciences).

Statistical Analyses

SPSS26 software was used for data analysis. The data were pre-sented as mean ± SEM. Statistical analyses were performed using paired or unpaired nonparametric tests (Mann–Whitney test, Krus-kal-Wallis test, Dunn's multiple comparison test and Spearman rank correlation). P value less than 0.05 was considered statistically significant.

Results

Patient General Information

A total of 44 subjects were included in the study as shown in Table 1, comprising 37 patients with CRSwNP, all diagnosed according to EPOS 2012 criteria. These patients were newly diagnosed and had no history of hormone use within the past month. The control group consisted of 7 patients with pituitary tumors without chronic sinusitis. The ages of the subjects ranged from 16 to 65 years, including 16 women and 21 men with CRSwNP, and 4 women and 3 men as controls. There was no significant difference in age and sex distribution between the CRSwNP patients and the controls.

Regarding the incidence, 17 participants had unilateral sinus disease, while 20 participants had bilateral sinus disease. Notably, during functional endoscopic sinus surgery (FESS), 15 patients underwent full sinus opening surgery. The percentage of peripheral blood eosinophils was significantly higher in patients with CRSwNP (7.43 ± 5.33) compared to controls (1.78 ± 1.25) ($P = 0.009$).

Increased Levels of IL-25 in NPs and TM Tissues of Patients with CRSwNP

Samples of NPs and TM from CRSwNP patients, as well as TM from the control group (patients with pituitary tumors without chronic sinusitis), were collected. Immunohistochemical methods were employed to detect the expression and distribution of IL-25 in these tissues. The results showed that IL-25 expression was significantly increased in the TM (Figure 1A) and NPs (Figure 1B) of CRSwNP patients compared to the TM of the control group (Figure 1C). The negative control (Figure 1D) was immuno-stained with isotype IgG to confirm the non-specific binding is low.

The percentage of the regional area of IL-25 in the TM was 1.52 ± 0.22 in the control group, 8.60 ± 1.04 in CRSwNP patients, and 12.32 ± 1.99 in the NPs of CRSwNP patients. This indicates that the IL-25 area percentage in both the TM andNPs of CRSwNP patients was significantly higher than that in the control group (Figure 1E, $P < 0.001$). Furthermore, the expression of IL-25 was significantly higher in the NPs than in the TM of CRSwNP patients (Figure 1E, $P < 0.01$).

Increased STAT3 Phosphorylation and Elevated Levels of Th2 Cytokines IL-5 and IL-13 Were Observed in CRSwNP-NP Compared to Control-TM

Nasal polyp (NP) tissues from CRSwNP patients and turbinate mucosa (TM) tissues from control subjects were subjected to protein extraction for p-STAT3 quantification. The levels of IL-5 and IL-13 in the extraction supernatant were measured by ELISA and compared between the CRSwNP and control groups.

As shown in Figure 2A and Figure S1 (uncropped image), p-STAT3 levels in NP tissues were significantly elevated compared to control TM tissues. Similarly, the production of Th2 cytokines IL-5 and IL-13 was markedly increased in NP tissues relative to control TM (Figure 2B and Table S1). Given the observed upregulation of IL-25 in NP tissues, it is reasonable to speculate that IL-25 may promote p-STAT3 activation, thereby contributing to the increased production of Th2 cytokines IL-5 and IL-13.

The Increasing Level of ILC2s in NPs and TM of CRSwNP Patients

As a key effector cell of IL-25, ILC2s are characterized by the markers $Lin^{-}FceR1^{-}CRTH2^{+}CD127^{+}$ and play a crucial role in promoting type 2 inflammation.[31] Our previous research has shown that ILC2s are predominantly responsible for the robust

Table 1 Baseline Characteristics of the Participants' Demographics, Blood Eosinophils Involved in This Study

Characteristic	CRSwNP	Control	*P* value
No. of patients	37	7	
Age (y)	38.14±13.78	35.83±15.12	0.6273
Gender, female/male	16/21	3/4	0.0681
Blood eosinophils (%)	7.43±5.33	1.78±1.25	0.009

Abbreviations: CRSwNP, Chronic sinusitis with nasal polyps; Control, Pituitary tumor patient without chronic sinusitis.

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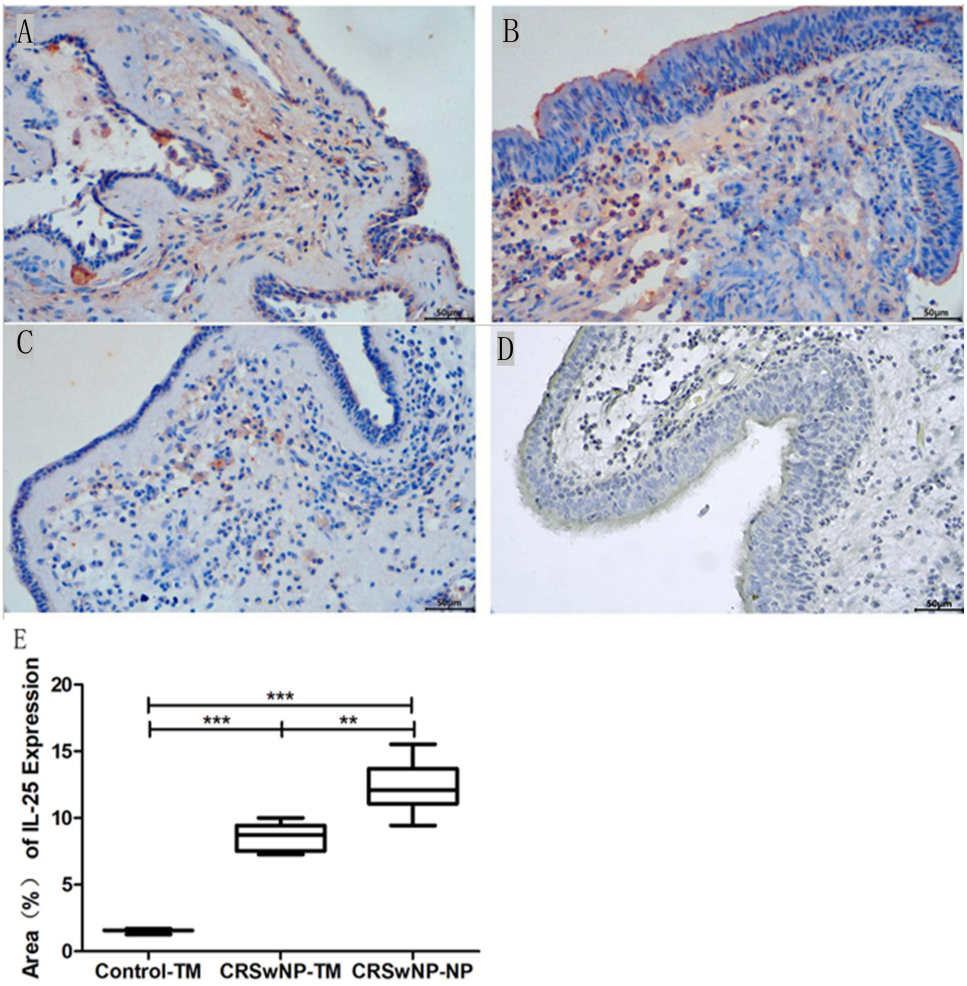

Figure 1 Expression of IL-25 in patients with CRSwNP and control patients. TM (**A**) and NP (**B**) tissues from patients with CRSwNP, and control TM from pituitary tumor patients without CRS (**C**) were immune-stained with IL-25 antibody. The negative control (**D**) was immunostained with isotype IgG. (**E**). Comparison of IL-25 expression levels in each tissue. **P < 0.01, ***P < 0.001.

production of Th2 cytokine IL-5, IL-13 and IL-9 in asthma with allergic rhinitis patients, a typical Type 2 inflammation.[29] And studies from Western countries suggest that ILC2s are highly elevated in eosinophilic NPs of CRSwNP patients.[28] However, the ILC2s remain under-explored in NPs and TM of CRSwNP patients in China. Thus, flow cytometry was used to measure ILC2s levels in NPs and TM of CRSwNP patients, with TM from non-CRS pituitary tumor patients serving as controls. A significant increase in ILC2s populations was observed in the NPs (0.4761 ± 0.2989%) and TM (0.1683 ± 0.1022%) of CRSwNP patients, whereas ILC2s were almost undetectable in the TM of the control group (0.0048 ± 0.0064%) (Figure 3A and Table S2, Scheme S1–S3). Although the ILC2s content was higher in NPs of CRSwNP patients, there was no statistically significant difference between the TM and NPs (Figure 3B and Table S2). The strong correlation between elevated ILC2s levels and increased IL-25 in NPs and TM suggests that IL-25-activated ILC2s cells play a pivotal role in the type-2 inflammation of CRSwNP Chinese Patients.

ILC2s Sorted from NPs Contribute to the Over-Production of Th2 Cytokine IL-5 and IL-13 Under IL-25 Stimulation

Although the proportion of ILC2s in NPs was higher than in the TM and control groups, they comprised less than 1% of total lymphocytes, making comprehensive cellular analysis challenging. ILC2s sorted from the NPs of six CRSwNP patients were collected and subjected to IL-25 stimulation and limonin treatment. As shown in Figure 4A, ILC2s exhibited the typical morphology of general lymphocytes. Upon IL-25 stimulation for 30 minutes, they increased in size (Figure 4B).

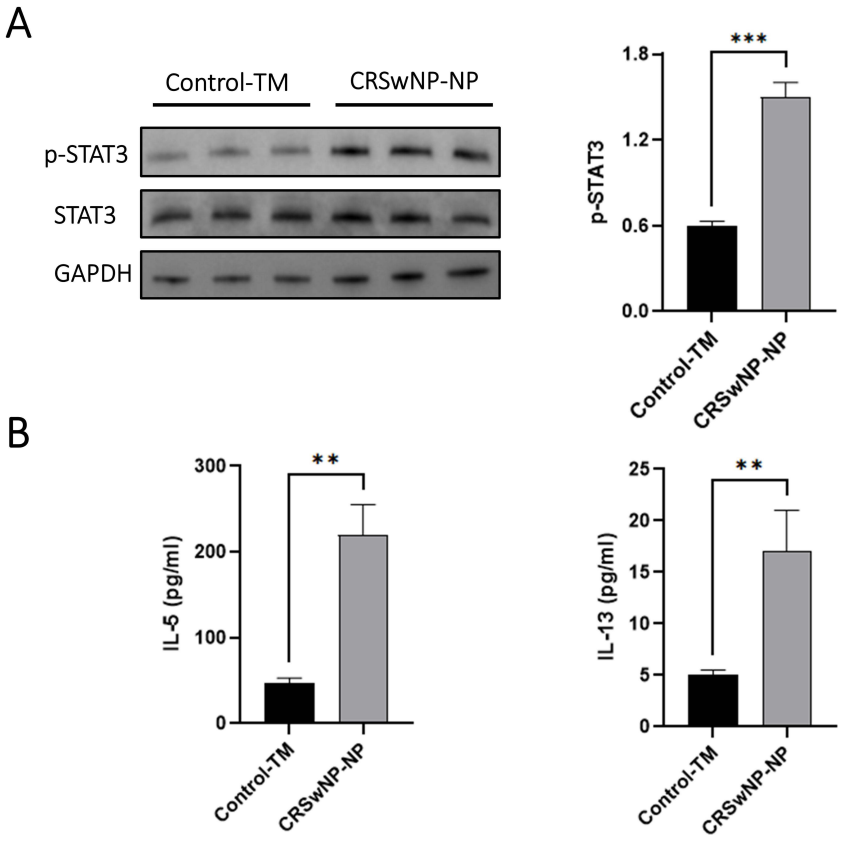

Figure 2 Phosphorylation of STAT3 and production of Th2 cytokines were elevated in CRSwNP-NP compared to control-TM. (**A**). Phosphorylation of STAT3 in NPs was upregulated compared to the control-TM. (**B**). The production of Th2 cytokines IL-5 and IL-13 in NPs were increased compared to the control-TM. **P < 0.01, ***P < 0.001.

Phosphorylated STAT3 (p-STAT3) levels rose significantly within 15 minutes, peaked at 30 minutes, and declined after 60 minutes (Figure 4C and Figure S2 for uncropped image). Additionally, IL-25 stimulation led to a significant increase in IL-5 (Figure 4F) and IL-13 (Figure 4G) production compared to untreated ILC2s (Table S3). These cytokines play a crucial role in recruiting and activating other immune cells, such as eosinophils, and contribute to mucus production and airway hyperreactivity—hallmarks of type-2 inflammatory diseases.

Limonin, a secondary metabolite found in citrus plants, has been widely studied for its anti-inflammatory properties.[32,33] Emerging research indicates that limonin and its derivatives can suppress inflammation by inhibiting the JAK-STAT and NF-κB signaling pathways.[34–37] Previous studies have shown that limonin can alleviate LPS-induced pulmonary dysfunction in mice by suppressing pro-inflammatory cytokine production through multiple signaling pathways, including activation of AMPKα/NRF2 and inhibition of NF-κB.[38,39] These findings underscore its therapeutic potential in airway inflammatory diseases. In this study, limonin treatment effectively reversed the p-STAT3 activation (Figure 4D and Figure S3 for uncropped image, Figure 4E), and inhibited the upregulation of IL-5 (Figure 4F) and IL-13 (Figure 4G) due to the IL-25 stimulation (Table S3). These findings suggest that ILC2s drive IL-5 and IL-13 overproduction via STAT3 signaling in NPs, highlighting limonin as a potential therapeutic strategy for managing type-2 inflammation in CRSwNP patients. This is the first report showing that limonin modulates ILC2s function by downregulating type 2 cytokine production, thereby attenuating inflammation in CRSwNP. Future work will focus on elucidating the broader immunomodulatory effects of limonin to further evaluate its potential as an anti-inflammatory agent in CRS.

Discussion

Our previous research has demonstrated that in peripheral blood mononuclear cells (PBMCs), there is an increase in eosinophils, which positively correlates with ILC2s levels in patients with asthma and allergic rhinitis (AR).[29] Study in the United States found that ILC2s are elevated in eosinophilic NPs but not in non-eosinophilic NPs and that the

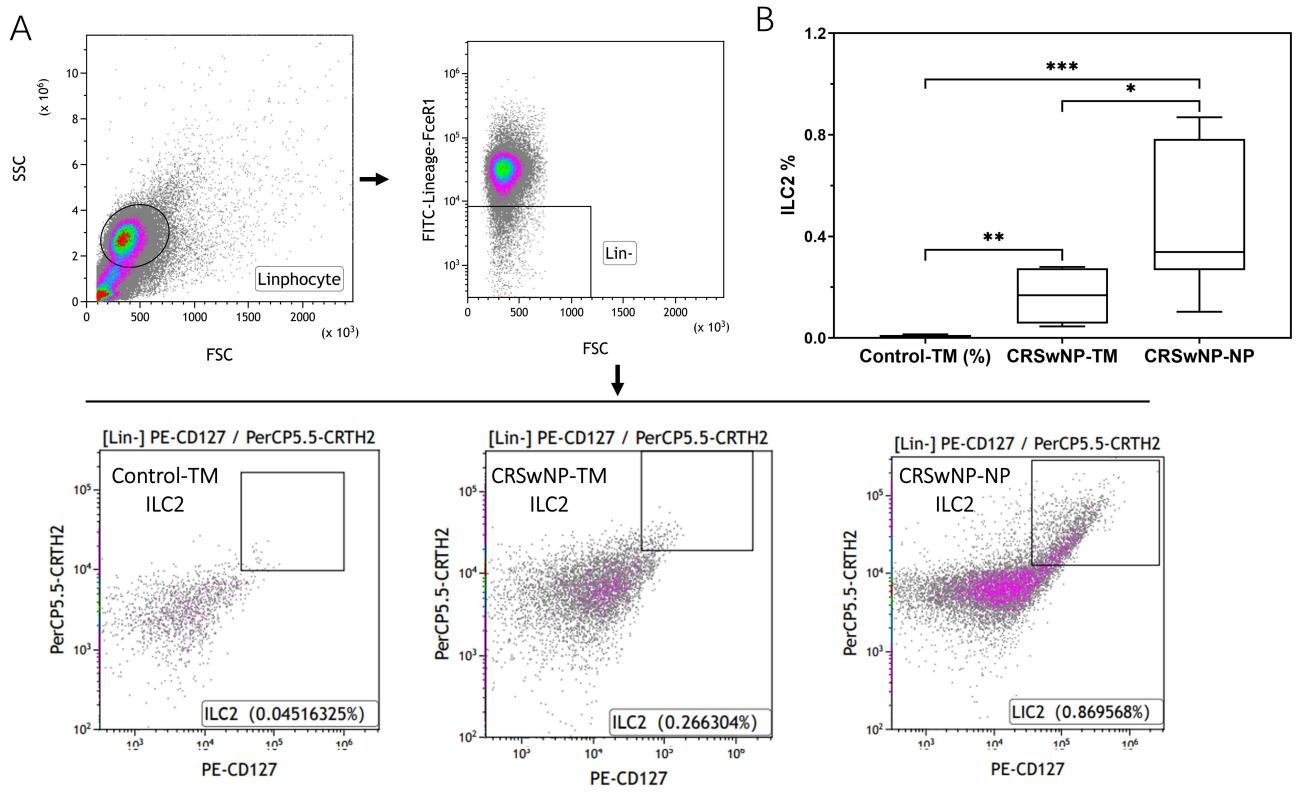

Figure 3 High ILC2s levels in CRSwNP patients in China. (**A**) Lymphocytes from nasal polyps (NP) and turbinate mucosa (TM) tissues were flow sorted, and ILC2s were defined as $Lin^{-}FceR1^{-}CRTH2^{+}CD127^{+}$ lymphocytes. Lineage-negative ($CD2^{-}$, $CD3^{-}$, $CD14^{-}$, $CD16^{-}$, $CD19^{-}$, $CD56^{-}$, $CD235a^{-}$) $FceR1^{-}$ cells were gated and further assessed for co-expression of CD127 and CRTH2 for control (**C**), CRSwNP TM (**D**) and CRSwNP NP (**E**), gating strategy is shown in Scheme S1 – S3. (**B**) ILC2s levels were quantified flow cytometry in control (n = 5), CRSwNP TM (n = 6), CRSwNP NP (n = 6) patients. *P < 0.05, **P < 0.01, ***P < 0.001.

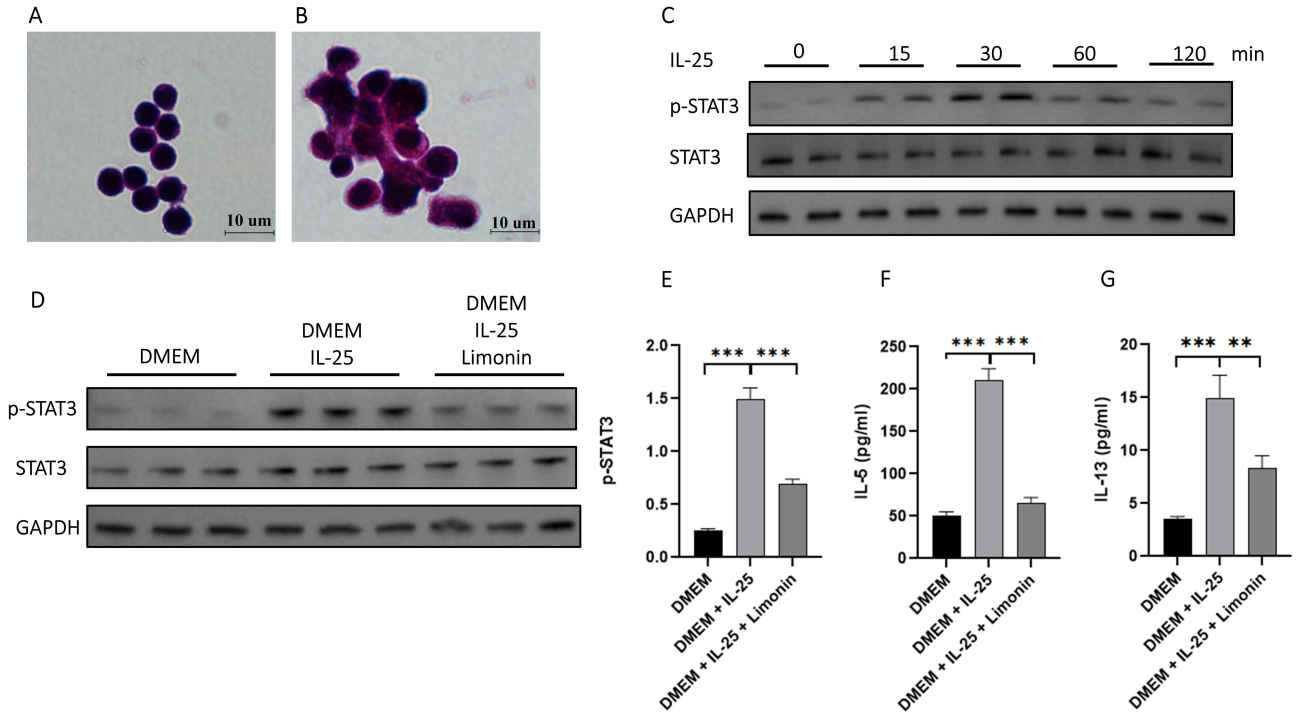

Figure 4 IL-25 Induces Morphological Changes in ILC2s and Enhances IL-5 and IL-13 Production through STAT3 pathway. (**A**) The morphology of ILC2s sorted from NPs of CRSwNP patients. (**B**) Morphological changes in ILC2s induced by IL-25 stimulation (10 ng/mL). (**C**) The phosphorylation of STAT3 in ILC2s was stimulated by treatment of IL-25 and peaked at 30 minutes. (**D** and **E**) The phosphorylation of STAT3 in ILC2s was inhibited by addition of limonin at 100 μM. (**F**) IL-5 production levels in ILC2s following IL-25 treatment, with the addition of limonin at 100 μM. (**G**) IL-13 production levels in ILC2s following IL-25 treatment, along with limonin at 100 μM. **P < 0.01, ***P < 0.001.

frequency of ILC2s positively correlates with eosinophils in NPs tissue.[40] In this study, we found that the percentages of peripheral blood eosinophils also show a positively correlation with ILC2s in NPs in Chinese patients.

IL-25, produced by infiltrating mast cells and nasal epithelial cells, plays a crucial role in the pathogenesis of type 2 inflammatory diseases such as asthma, atopic dermatitis, and CRSwNP.[20,41] Our findings further confirmed that IL-25 is overexpressed in NPs and TM tissues from Chinese CRSwNP patients. Besides, p-STAT3 levels in NP tissues were significantly elevated compared to control TM tissues. Similarly, the production of Th2 cytokines IL-5 and IL-13 was markedly increased in NP tissues relative to control TM (Figure 2B). Given the observed upregulation of IL-25 in NP tissues, it is reasonable to speculate that IL-25 may promote p-STAT3 activation, thereby contributing to the increased production of Th2 cytokines IL-5 and IL-13.

Various cell types, including ILC2s, macrophage cells and mast cells in human NPs, have shown a strong correlation with IL-25 expression.[31,42,43] In this study, ILC2s were found to have increased abundance in the NPs and TM tissues of Chinese patients with CRSwNP. In contrast, TM tissues from patients with pituitary tumors exhibited a low content of ILC2s. The results suggest that ILC2s play a key role in regulating the inflammation in NPs from Chinese patients.

Studies have shown that ILC2s are elevated in NPs and contribute to the type 2 inflammatory response induced by epithelial-derived innate cytokines.[28] This study explored the role of ILC2s in NP tissues of Chinese CRSwNP patients in response to IL-25. In vitro stimulation with IL-25 increased ILC2 size and p-STAT3 levels, leading to the overexpression of IL-5 and IL-13. Notably, limonin inhibited STAT3 phosphorylation and reduced IL-5 and IL-13 production, highlighting ILC2s as key IL-25 effector cells in NPs and suggesting limonin as a potential therapeutic for CRSwNP by targeting p-STAT3. However, the low abundance of ILC2s in NPs presents challenges for systematic study. Future work will focus on collecting and analyzing ILC2s from NP tissues of Chinese CRSwNP patients to further investigate their role.

In conclusion, this study identified ILC2s as key effector cells of IL-25 in NPs from Chinese CRSwNP patients. IL-25 and ILC2 levels were significantly elevated in the NPs and TM of these patients. Upon IL-25 stimulation, ILC2s in NPs contributed to the overexpression of Th2 cytokines IL-5 and IL-13 via the STAT signaling pathway. Notably, limonin inhibited STAT3 phosphorylation, thereby reducing IL-5 and IL-13 production, highlighting its potential as a therapeutic strategy for controlling type-2 inflammation in Chinese CRSwNP patients.

Highlights

1. IL-25 stimulates NPs tissues of Chinese CRSwNP patients to produce Th2 cytokines IL-5 and IL-13 through STAT3 pathway which are the key drivers for type 2 inflammation.
2. ILC2s sorted from NPs of Chinese CRSwNP patients significantly produce IL-5 and IL-13 via STAT3 pathway.
3. ILC2s function as potential effector cells of IL-25 in NPs in Chinese CRSwNP patients.
4. Limonin can Inhibit the phosphorylation of STAT3 suppressing the production of Th2-type cytokines IL-5 and IL-13 in ILC2s, which demonstrate the potential to control the type 2 inflammation of CRSwNP.

Data Sharing Statement

Raw data of Western Blot, ELISA and gating strategy of flow cytometry analysis are in the supporting material. Original fcs files of flow cytometry are available upon reasonable request to the corresponding authors, Drs. Shaochi Wang (shaochiwang127@zzu.edu.cn, wangshaochi1990@hotmail.com) and Yulin Zhao (zhaoyulinmail@163.com).

Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of The First Affiliated Hospital of Zhengzhou University (2024-KY -0405-002). Written informed consent was obtained from 44 subjects (ages 16 – 65).

Human Ethics Declaration

This study was conducted in accordance with the ethical guidelines outlined in the Institutional Review Board (IRB), Ethics Committee of The First Affiliated Hospital of Zhengzhou University. Ethical approval was obtained from Ethics Committee of The First Affiliated Hospital of Zhengzhou University, and all research procedures adhered to the principles of the Declaration of Helsinki.

Consent to Participate

All participants were informed about the study's objectives, procedures, potential risks, and benefits before participation. Written informed consent was obtained from all participants (or from their legal guardians if they were minors) before their involvement in the study. Participants were assured that their participation was voluntary and that they could withdraw at any time without any consequences.

Consent for Publication

All the authors have reviewed the manuscript and agreed to publish it.

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Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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