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ORIGINAL RESEARCH

The Role of Type 2 Innate Lymphoid Cells in Adenoid Hypertrophy with Allergic Rhinitis Among Children and Related Potential Therapeutic Targets

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Objective: Innate lymphoid cells (ILC) are a heterogeneous group of immune cells implicated in immune diseases. However, their specific roles in adenoid hypertrophy (AH), AH with allergic rhinitis (AH+AR), and AH with otitis media with effusion (AH+OME) remain poorly understood. This study aimed to characterize ILC subsets and their association with immunological profiles in these conditions.

Methods: Flow cytometry was used to quantify ILC subsets in adenoid tissues from patients with AH, AH+AR, or AH+OME, and correlations between ILC subsets and clinical, immunological (serum and tissue cytokines), and histopathological parameters were further assessed.

Results: ST2 mRNA and protein expression were significantly higher in AH+AR than in AH and AH+OME ($p < 0.05$). Serum IL-33 was elevated in AH+AR compared to AH ($p = 0.0127$), while IFN-γ was higher in AH than in AH+AR ($p = 0.0044$). IL-4 levels were higher in AH and AH+AR than in AH+OME ($p < 0.005$). Flow cytometry showed that ILC2 predominated in AH+AR ($p = 0.0009$ vs AH+OME), with higher ILC2/ILC1 and ILC2/ILC3 ratios in AH and AH+AR compared to AH+OME ($p < 0.05$). Correlation analysis indicated that ILC2 in AH+AR positively correlated with serum IgE, IL-4, IL-33, thymic stromal lymphopoietin (TSLP), and tissue IL-4, IL-33, TSLP, and IL-25 ($p < 0.05$). ILC3 inversely correlated with peripheral blood eosinophils ($p = 0.0125$) and positively with serum and tissue IL-17A ($p < 0.05$).

Conclusion: ILC2 cells are significantly enriched in adenoid tissues of patients with AH+AR, with elevated ST2 and IL-33 levels supporting the activation of the IL-33/ST2/ILC2 signaling pathway. Targeting this pathway may offer novel therapeutic strategies for AH combined with allergic rhinitis.

Keywords: adenoid hypertrophy, innate lymphoid cells, allergic rhinitis, otitis media with effusion, group 2 innate lymphoid cell

Introduction

Adenoid hypertrophy (AH), characterized by excessive enlargement of the pharyngeal tonsils, is a common pediatric condition affecting up to 34.5% of children.[1,2] Its clinical manifestations, including nasal obstruction, snoring, and sleep-disordered breathing, can have profound impacts on children's health and development, potentially leading to serious consequences such as maxillofacial deformities, cognitive impairments, and even cardiovascular issues.[3,4] While adenoidectomy remains the primary treatment for symptomatic AH, it carries inherent risks and may not be suitable for all patients, particularly those with AH and allergic rhinitis (AH+AR) facing a higher risk of postoperative recurrence.[5–7] This underscores the critical need for a better understanding of the underlying pathophysiology of AH to develop more targeted and effective treatment strategies.

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The adenoid, a key component of Waldeyer's ring, serves as a crucial immune barrier in the upper respiratory tract, harboring a diverse array of immune cells, including lymphocytes, macrophages, and dendritic cells.[8] While the exact mechanisms driving AH remain elusive, chronic exposure to allergens and pathogens is widely believed to play a significant role in its pathogenesis.[9] Upon encountering antigens, resident antigen-presenting cells within the adenoid initiate immune responses by activating naïve T and B lymphocytes, leading to the differentiation of effector cells that mediate immune responses through direct cell-to-cell interactions or the secretion of cytokines.[3,10]

While the role of adaptive immunity in AH has been investigated, the contribution of innate immunity, particularly innate lymphoid cells (ILC), remains largely unexplored.[11–13] Innate lymphoid cells, a heterogeneous group of innate immune cells, are classified into three major subsets based on their cytokine profiles: ILC1 (producing IFN-γ), ILC2 (producing IL-5, IL-13), and ILC3 (producing IL-17A and/or IL-22).[14] These ILC subsets exhibit functional similarities to T helper (Th) cell subsets, playing crucial roles in various immune responses, including mucosal immunity. Given the pivotal role of innate immunity, especially in early childhood when the adaptive immune system is still developing, ILCs are likely to play a significant role in the pathogenesis of AH.

This study aims to identify potential therapeutic targets for AH therapy based on the investigation of the distribution and function of ILC subsets in adenoid tissues by characterizing ILC subsets and assessing their correlations with clinical parameters, inflammatory cytokines, and other immunological markers.

Methods and Materials

Patients and Tissue Collection

Adenoid tissue samples were obtained from pediatric patients undergoing adenoidectomy at Shenzhen Longgang Otolaryngology Hospital from September 2021 to September 2023. Inclusion was limited to patients diagnosed with adenoid hypertrophy (AH). Exclusion criteria were parasitic or viral infections, primary immunodeficiency, recent steroid use (oral or nasal within the last 3 months), antileukotrienes or antibiotics (within 4 weeks pre-surgery), desensitization therapy, and inadequate tissue yield (<200 μL). AH severity was graded using the nasal endoscopic quadrature method by Franco et al.[15] with obstruction levels categorized as: 1 (0–25%), 2 (26–50%), 3 (51–75%), and 4 (76–100%). Informed consent was obtained from parents or guardians, and the study was approved by the Ethics Committee of Shenzhen Longgang Otolaryngology Hospital (ZSSOM 2021–0910) and conducted in accordance with the Declaration of Helsinki.

Mononuclear Cell Isolation and Multicolor Flow Cytometry

Adenoid tissues were minced and filtered through a 70 μm sieve. Cells were washed with PBS, centrifuged at 400g for 5 minutes at 4°C, and the pellet resuspended in PBS. Mononuclear cells were isolated using Ficoll density gradient centrifugation (GE Healthcare) and adjusted to 10^7 cells/mL. The cells were stained with a panel of antibodies: PE anti-human CD1a (BioLegend, Cat# 300105), CD123 (BioLegend, Cat# 396604), CD14 (BioLegend, Cat# 325606), CD19 (BioLegend, Cat# 302208), CD20 (BioLegend, Cat# 302306), CD3 (BioLegend, Cat# 300308), CD303 (BioLegend, Cat# 354204), FcεRIα (BioLegend, Cat# 334610), CD235a (BioLegend, Cat# 349106), CD66b (BioLegend, Cat# 392904), CD34 (BioLegend, Cat# 343606); FITC anti-human CD45 (BioLegend, Cat# 304006); APC anti-human CD336 (NKp44) (BioLegend, Cat# 325110); FITC anti-human CD117 (c-kit) (BioLegend, Cat# 313226); APC/Cyanine7 anti-human CD161 (BioLegend, Cat# 339928); PerCP/Cyanine5.5 anti-human CD127 (IL-7Rα) (BioLegend, Cat# 351322); and PE/Cyanine7 anti-human CD294 (CRTH2) (BioLegend, Cat# 350118). Cells were stained with a panel of antibodies for 30 minutes at room temperature, protected from light, then analyzed using a BD FACSAria™ II flow cytometer (BD Biosciences).

Immunohistochemistry

Paraffin-embedded sections were dewaxed and subjected to antigen retrieval. After blocking endogenous peroxidase activity, sections were incubated with primary and secondary antibodies for ST2, followed by tyramide signal amplification. Similar procedures were used for TSLP and IL-25R antibodies. The positive tissue sections were microscopically brown or yellowish brown, and tissue images were taken by a Nikon (ECLIPSE Ci-L). Three views of each slice were

randomly selected. Image J software was used to measure the integrated optical density (IOD) of tissue sections, and the average IOD (AOD) values were considered as the relative expression of proteins.

RT-qPCR Assay

Total RNA was extracted using Trizol reagent (Invitrogen), and cDNA was synthesized using the PrimeScript™ RT Reagent Kit (TaKaRa). RT-qPCR was performed on a 7500 Real-Time PCR System using the SYBR® Premix Ex Taq™ II kit (TaKaRa), with primer sequences listed in Table 1.

Cytokines Measurement

Serum and adenoid tissue homogenates were assayed for cytokine levels using ELISA kits (DAKEWE), following the manufacturer's instructions.

Western Blot Analysis

Tissues were homogenized in RIPA buffer and proteins were separated on SDS-polyacrylamide gels, transferred to PVDF membranes, and blocked. Membranes were incubated with primary antibodies (ST2, TSLPR, IL-25R, RORγt, T-bet, GATA3, GAPDH, Cell Signaling Technology) and HRP-conjugated secondary antibodies. Signals were detected using an enhanced chemiluminescence system (Thermo Fisher Scientific).

Statistical Analysis

Data were analyzed using one-way ANOVA with Bonferroni's post hoc test and Pearson's correlation analysis. GraphPad Prism software (version 9.0) was used, with $p < 0.05$ considered significant.

Results

Clinical Characteristics of the Study Population

A total of 40 patients were enrolled in the adenoid hypertrophy (AH) group, 40 in the AH with allergic rhinitis (AH+AR) group, and 25 in the AH with otitis media with effusion (AH+OME) group to assess innate lymphoid cell (ILC) subsets in adenoid tissues. Demographic and clinical data are summarized in Table 2. The AH+AR group exhibited significantly higher serum total IgE (214.7 ± 31.91 IU/mL) and blood eosinophil levels (0.3650 ± 0.0382%) compared to the AH group (IgE: 85.52 ± 16.51 IU/mL, eosinophils: 0.2375 ± 0.0281%). Monocyte levels were also elevated in the AH+OME group (0.3976 ± 0.0243%). No significant differences in age or blood basophil and neutrophil levels were observed between the groups.

Distribution of ILC Subsets in AH, AH+AR, and AH+OME

Adenoid tissues were processed into single-cell suspensions for flow cytometry analysis. To identify distinct subsets of ILCs, a 7-color flow cytometry panel was employed, using the following antibodies: PE anti-human lineage cocktail

Table 1 Primer Sequences

Primer		Sequences (5'-3')	Primer		Sequences (5'-3')
IL-33	F	GTGACGGTGTTGATGGTAAGAT	TLSPR	F	AGTGACGGTGACGTGTTCTG
	R	AGCTCCACAGAGTGTTCCTTG		R	CTATGGTGACGTTGCAGGTATT
IL-25	F	CAGGTGGTTGCATTCTTGGC	IL25R	F	CAGGTGGTTGCATTCTTGGC
	R	GAGCCGGTTCAAGTCTCTGT		R	GAGCCGGTTCAAGTCTCTGT
TSLP	F	AGTGACGGTGACGTGTTCTG	T-bet	F	GGTTGCGGAGACATGCTGA
	R	CTATGGTGACGTTGCAGGTATT		R	GTAGGCGTAGGCTCCAAGG
ST2	F	ATGGGGTTTTGGATCTTAGCAAT	GATA3	F	GCCCCTCATTAAGCCCAAG
	R	CACGGTGTAACTAGGTTTTCCTT		R	TTGTGGTGGTCTGACAGTTCG
GAPDH	F	GGCATGGACTGTGGTCATGAG	RoRγt(Rorc)	F	GTGGGGACAAGTCGTCTGG
	R	TGCACCACCAACTGCTTAGC		R	AGTGCTGGCATCGGTTTCG

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Table 2 Preoperative Demographic Characteristics of Subjects

Characteristics	AH (n = 40)	AH+AR (n = 40)	AH+OME (n = 25)
Age (y), median (range)	5.5 (2–11)	4(3–12)	4(3–12)
Male/female	21/19	25/15	19/6
AH grade	25(III)/15(IV)	21(III)/19(IV)	14(III)/11(IV)
Serum total IgE (IU/mL), (Mean±SEM)	85.52±16.51	214.7±31.91*	135.3±35.27
Blood eosinophils (%), (Mean±SEM)	0.2375±0.0281	0.3650±0.0382*	0.2824±0.0311
Blood basophils %l), (Mean±SEM)	0.0515±0.0058	0.0485±0.0035	0.0392±0.0051
Blood monocytes (%), (Mean±SEM)	0.2915±0.0211	0.3393±0.0204	0.3976±0.0243*
Blood neutrophils (%), (Mean±SEM)	3.893±0.2031	4.230±0.3201	3.643±0.2151

Note: *p<0.05 indicate the significant difference to AH group.

(CD1a, CD123, CD14, CD19, CD20, CD3, CD303, FcεRIα, CD235a, CD66b, CD34); FITC anti-human CD45; APC anti-human CD336; FITC anti-human CD117; APC/Cyanine7 anti-human CD161; PerCP/Cyanine5.5 anti-human CD127; and PE/Cyanine7 anti-human CD294. After quality control, $CD45^+Lin^-CD127^+$ cells were categorized into three ILC subsets based on the expression of CD294 (CRTH2), CD117, and CD161: ILC1 ($CD45^+Lin^-CD127^+CD294^-CD117^-CD161^+$), ILC2 ($CD45^+Lin^-CD127^+CD294^+$), and ILC3 ($CD45^+Lin^-CD127^+CD294^-CD117^+$) (Figure 1).

Cytokine and Alarmin Levels in Peripheral Blood and Adenoid Tissue

ELISA analysis revealed significantly elevated serum IL-4 levels in the AH+AR group (34.78±3.45 pg/mL, p <0.001) and AH group (27.81±4.08 pg/mL, p = 0.0034) compared to the AH+OME group (9.73±1.63 pg/mL) (Figure 2E). Serum IFN-γ levels were significantly higher in the AH group (4.86±0.45 ng/mL, p = 0.0044) than in the AH+AR group (3.24±0.20 ng/mL) (Figure 2D). No significant differences were observed in tissue IFN-γ or IL-4 levels among the three groups. Adenoid tissue IL-33 levels were significantly higher in the AH+AR group (1020.00 ±57.28 pg/mL, p = 0.0127) compared to the AH group (826.00±33.43 pg/mL) (Figure 2G). Although serum IL-33 levels tended to be higher in the AH+AR group (568.90±97.25 pg/mL) compared to the AH group (488.70±85.87 pg/mL) and AH+OME group (287.00±64.38 pg/mL) (Figure 2A), these differences did not reach statistical significance.

Correlation of ILC Subsets with Cytokine Levels

In the AH+OME group, ILC1 exhibited a significant negative correlation with serum IgE levels (p = 0.0178, Figure 3I). ILC2 showed significant positive correlations with serum IgE (p = 0.0017, Figure 3A), serum IL-4 (p = 0.0013, Figure 3C), serum IL-33 (p = 0.0123, Figure 3E), serum TSLP (p = 0.0200, Figure 3H), and tissue levels of IL-4 (p = 0.0007, Figure 3C), IL-33 (p = 0.0002, Figure 3F), IL-25 (p = 0.0477, Figure 3D), and TSLP (p < 0.0001, Figure 3H) in the AH+AR group. ILC3 demonstrated a significant positive correlation with serum IL-17A (p = 0.0401, Figure 3J) and tissue IL-17A (p = 0.0131, Figure 3K) in the AH+OME group, and a significant negative correlation with peripheral blood eosinophils (p = 0.0125, Figure 3L) in the AH+AR group.

Expression of Alarmin Receptors in Adenoid Tissue

Immunohistochemistry (IHC) analysis indicated higher expression of alarmin receptors ST2, in the AH+AR group's adenoid tissue compared to the AH groups (p=0.0039, Figure 4B). Western blot and RT-qPCR confirmed these findings, showing increased IL-33 and ST2 levels in the AH+AR group (Figure 5A–L). ST2 protein expression was higher than AH group (p = 0.0371, Figure 5A and B). ST2 mRNA expression was significantly higher in the AH+AR group than in the AH (p = 0.0002, Figure 5L) and AH+OME (p = 0.0051, Figure 5L) groups. Conversely, the transcription factor GATA3 was more highly expressed in the AH group compared to the AH+AR group (p = 0.0011, Figure 5P).

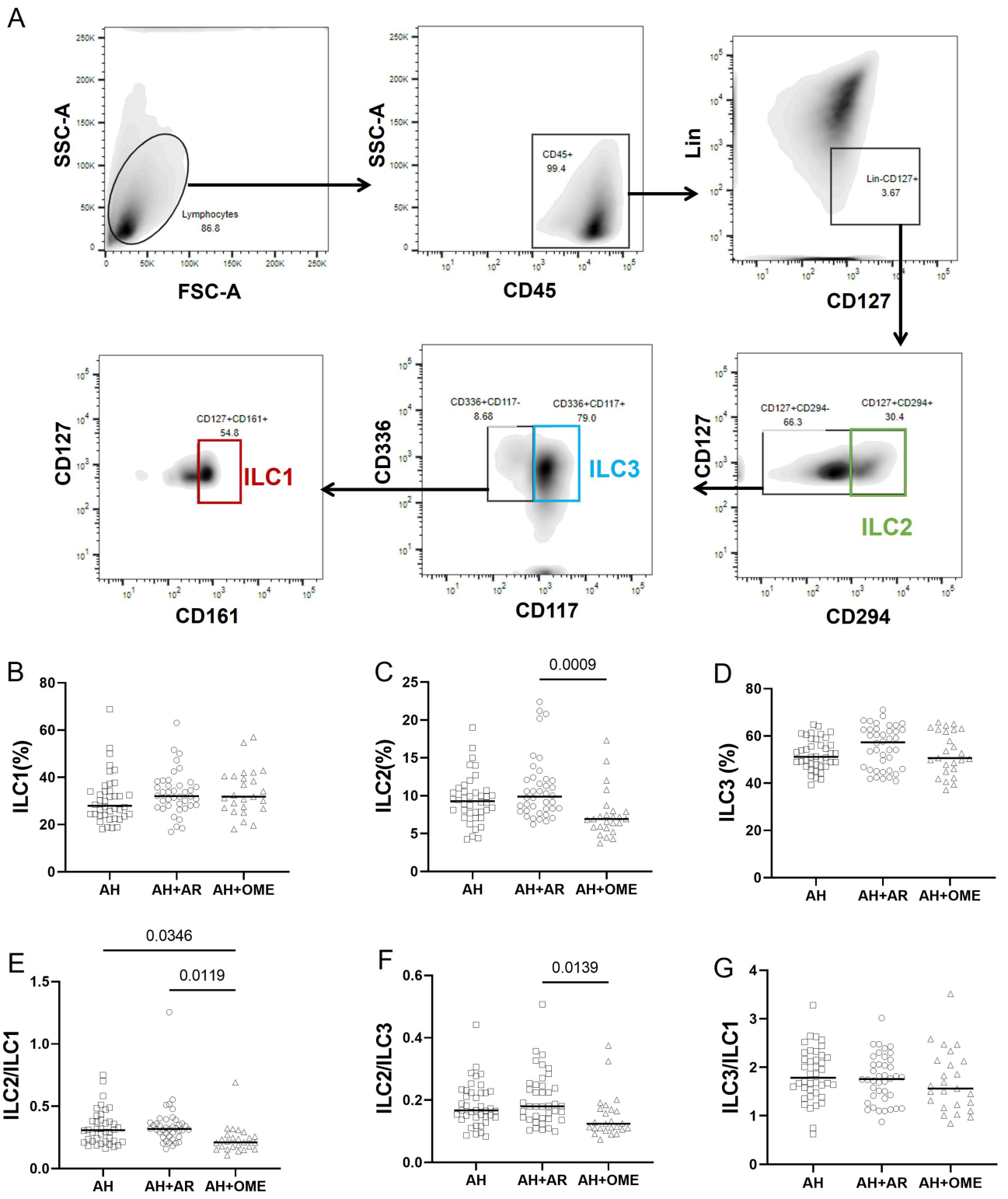

Figure 1 Flow cytometric analysis of ILC1, ILC2, and ILC3 in adenoid tissue (**A**). The AH+AR group exhibited a significantly higher proportion and number of ILC2 cells compared to the AH+OME group ($p = 0.0009$, **C**). No significant differences were found in ILC1 (**B**) and ILC3 (**D**) cell populations among the three groups. The ILC2/ILC1 ratio was significantly elevated in the AH ($p = 0.0346$, **E**) and AH+AR ($p = 0.0119$, **E**) groups relative to the AH+OME group. Additionally, the ILC2/ILC3 ratio was significantly higher in the AH+AR group compared to the AH+OME group ($p = 0.0139$, **F**). (**G**) No significant differences in ILC2/ILC1 ratio among the AH, AH+AR, and AH+OME groups.

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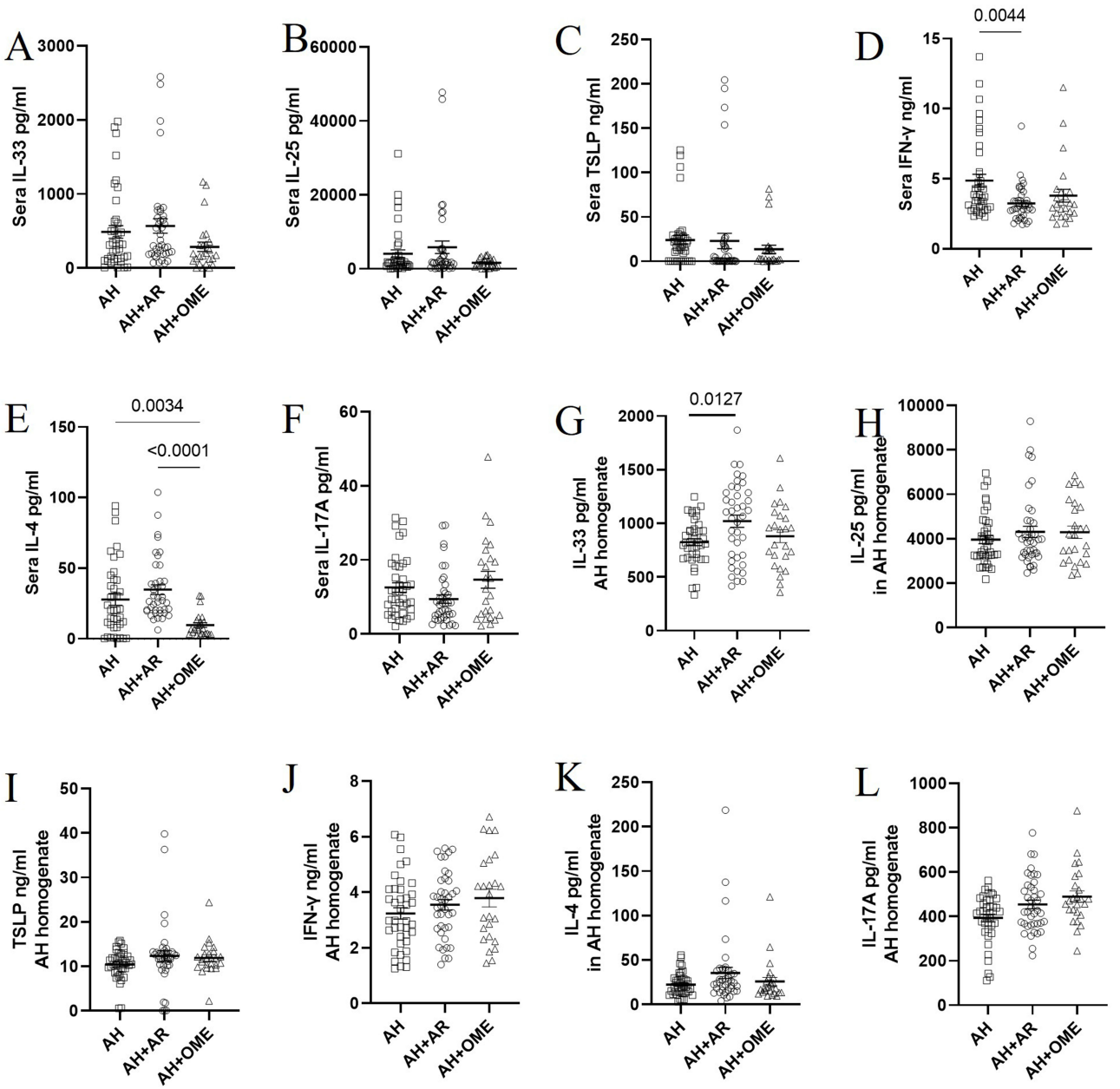

Figure 2 Expression of cytokines in the peripheral blood and homogenate of adenoid tissue in AH, AH+AR group and AH+OME group. The sera cytokines were determined by ELISA (**A**–**L**). The AH+AR showed high levels of IL-33, IL-25 and TSLP in peripheral blood and homogenate of adenoid tissue than those in AH and AH +OME, but there was no significant difference among these three groups (**A**–**C**, **G**–**I**). Sera IFN-γ in AH is higher than that in AH+AR group (p =0.0044, (**D**) and sera levels of IL-4 in AH group (p = 0.0035, **E**) and AH+AR group (p =0.0083, **E**) were higher those in AH+OME. IL-33 in homogenate in AH group was higher than that in AH group.

Discussion

Adenoid tissue plays a crucial role in the host defense of the upper respiratory tract against invading antigens. Lymphoid tissue undergoes significant postnatal development, reaching its maximum size during early childhood and subsequently undergoing involution around the age of 7–10 years.[16] These tissues possess four distinct histological compartments: the follicular germinal center, the mantle zone, the interfollicular area, and the crypt epithelium, all of which are critical for immune responses.[17] Germinal center reactions are characterized by rapid clonal expansion of B cells, spatially organized into two distinct zones. The dark zone, enriched with proliferating centroblasts, is proximal to T lymphocytes, while the light zone harbors centrocytes, which are non-proliferating B cells. T lymphocytes provide essential help to B cells through CD40-CD40L interactions and cytokine production. Conversely, B lymphocytes present processed antigens (pMHCII) to T cells,

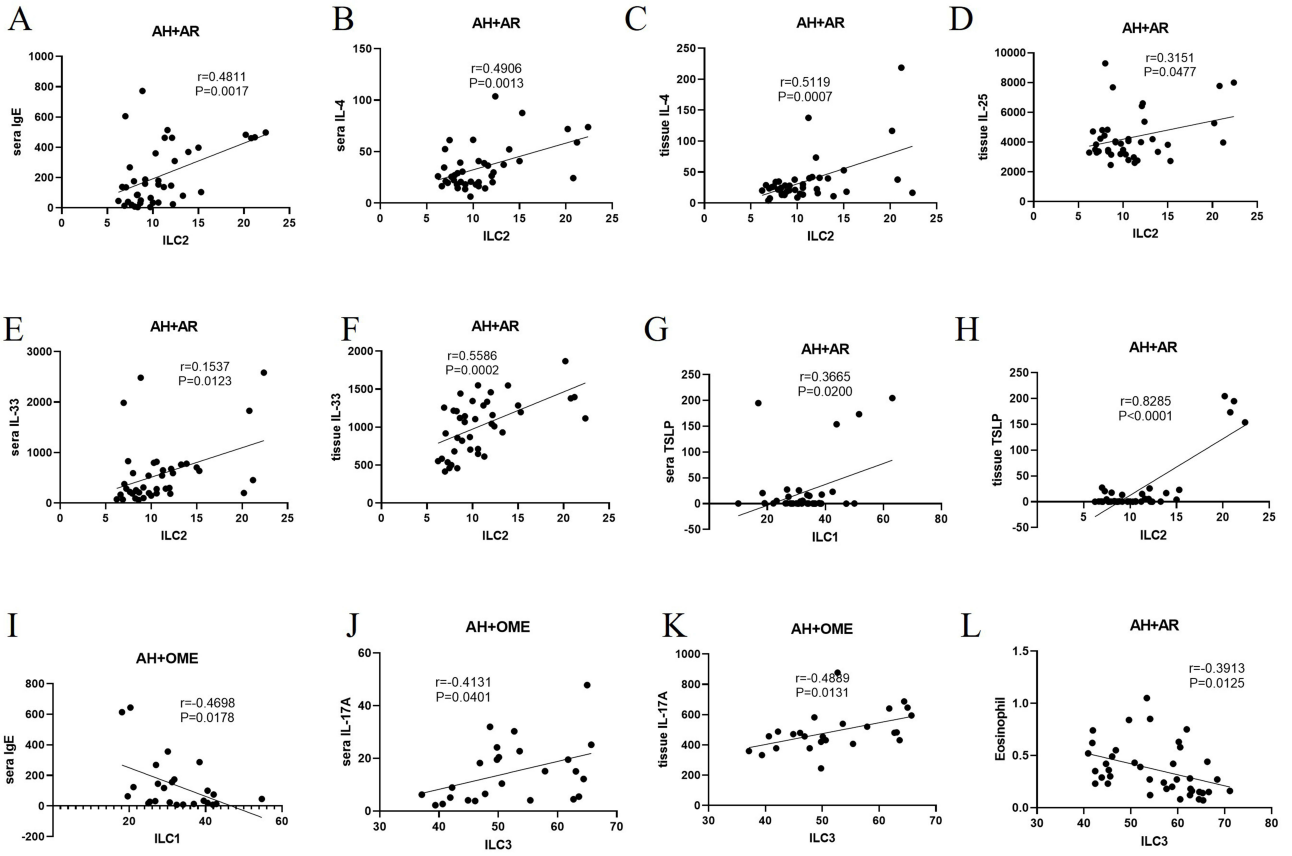

Figure 3 Correlation of ILCs subset with cytokines in the peripheral blood and homogenate of adenoid tissue in AH group, AH+AR group and AH+OME group. ILC1 showed a positive correlation with sera TSLP (**G**) and negative association with sera IgE in AH+OME group (p =0.0178, **I**). ILC2 was correlated with sera IgE (p = 0.0017, **A**), sera IL-4 (p = 0.0013, **B**), sera IL-33 (p = 0.0123, **E**), sera TSLP (p = 0.0200, **H**), and IL-4 in tissue of adenoid (tissue IL-4, p = 0.0007, **C**), tissue IL-33 (p = 0.0002, **F**), tissue IL-25 (p = 0.0477, **D**), tissue TSLP (p < 0.0001, **H**) in AH+AR group. ILC3 positively associated with sera IL-17A (p = 0.0401, **J**) and tissue IL-17A (p = 0.0131, **K**) in AH +OME group, and natively with peripheral blood eosinophils (p = 0.0125, **L**) in AH+AR group.

stimulating their activation and cytokine secretion. Dendritic cells (DCs) are strategically positioned between T and B cell zones.[18] These antigen-presenting cells play a crucial role in directing the polarization of the immune response towards Th1 or Th2 pathways, a critical step in the development of middle ear inflammatory processes.[19]

The etiology of AH in children remains elusive, although it is likely multifactorial, involving a complex interplay of immune, hormonal, and genetic factors.[20] Frequent upper respiratory tract infections and allergies are recognized as significant risk factors.[21,22] Bacterial infections are common, with *Haemophilus influenzae, Streptococcus pneumoniae, Streptococcus pyogenes, and Staphylococcus aureus* being frequently isolated from adenoid tissue. Chronic infections may involve the presence of anaerobic bacteria.[23] Additionally, exposure to gastric juice during gastroesophageal reflux disease can contribute to AH, particularly in infants and young children.[24] Previous studies have identified four distinct inflammatory endotypes: Th1, Th17, neutrophil-dominant with type 2 features, and type 2 feature. Serum levels of total IgE (TIgE) have been identified as a valuable biomarker for predicting the specific endotype of adenoid inflammation.[25] An imbalance in the Th17/Treg cell ratio may increase the risk of developing obstructive sleep apnea (OSA), and allergic rhinitis (AR) may further exacerbate this risk.[26] Furthermore, decreased ratios of Th17/Treg subpopulations have been implicated in the pathogenesis of adenoid hypertrophy.[27] Low vitamin D levels and reduced frequencies of regulatory T cells (Tregs) have also been associated with adenotonsillar hypertrophy in children.[28]

Previous research on the pathogenesis of AH has predominantly focused on adaptive immune responses and IgE-mediated allergic reactions, with particular emphasis on the roles of Treg, Th17, and Breg cells.[3,12,13] In contrast, non-IgE-mediated disorders, also characterized as cell-mediated or delayed-type hypersensitivity reactions, are primarily driven by other immune cells, such as innate lymphoid cells (ILCs).[29] There is increasing recognition that non-IgE-

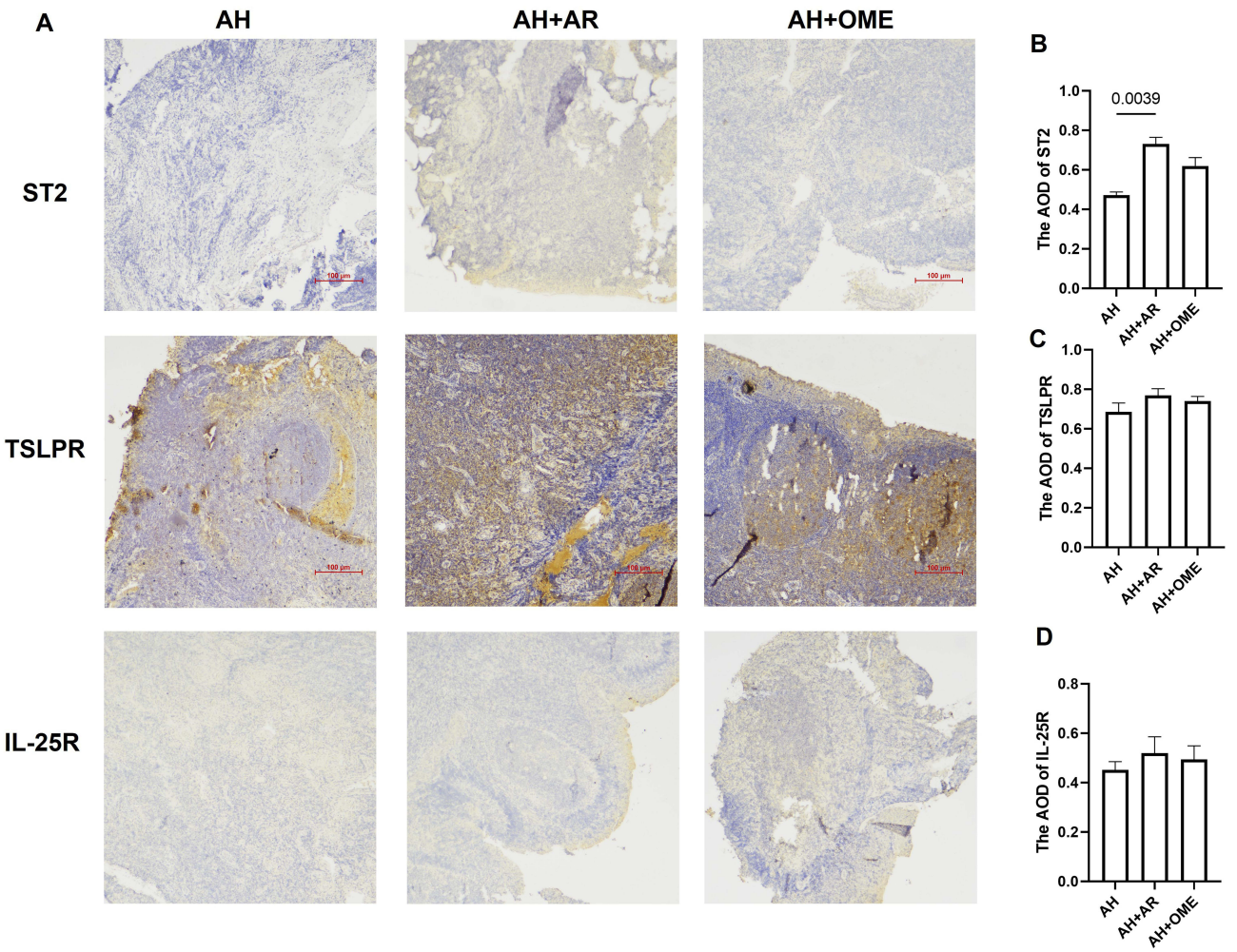

Figure 4 Expression of alarmin receptors in adenoid tissue of AH, AH+AR, and AH+OME. Panel (**A**) showed representative IHC staining of ST2, TSLPR, and IL-25R in adenoid tissue from the AH, AH+AR, and AH+OME groups. The integrated optical density (IOD) for ST2 was significantly higher in the AH+AR group compared to the AH group ($p = 0.0039$, **B**). The IOD for TSLPR (**C**) and IL-25R (**D**) in adenoid tissue sections from each group is also presented.

mediated mechanisms can also play a crucial role in immune hypersensitivity diseases.[30,31] Our study expands this understanding by investigating the role of ILCs in AH, specifically examining their involvement in AH with allergic rhinitis (AH+AR) and AH with otitis media with effusion (AH+OME). Our findings demonstrate a significant enrichment of ILC2 cells in the adenoid tissues of patients with AH+AR compared to those with AH+OME, suggesting a strong association between ILC2 and the development of allergic manifestations in AH. This observation aligns with the growing body of evidence supporting the crucial role of type 2 immunity in allergic diseases.[32,33] The elevated expression of ST2, the receptor for IL-33, in the adenoid tissues of AH+AR patients further strengthens this notion. IL-33, a potent alarmin released by epithelial cells and other damaged tissues, is a key activator of type 2 immune responses, including the activation and recruitment of ILC2. The observed positive correlation between ILC2 and serum IgE, IL-4, and other Th2 cytokines in AH+AR further emphasizes the contribution of ILC2 to the Th2-biased immune response characteristic of allergic inflammation. Our study highlights the significant contribution of innate immunity, particularly ILC2, in the development of AH+AR. This finding addresses a critical gap in our understanding of AH by demonstrating the heightened expression of ILC2 in the AH+AR group compared to the AH+OME group, suggesting that ILC2 play a pivotal role in driving the allergic phenotype observed in this condition.

The pathogenesis of AH with OME is a complex process. Risk factors for AH+OME in pediatric patients include young age, high adenoid grade, exposure to environmental tobacco smoke (ETS), non-breastfeeding status, comorbid

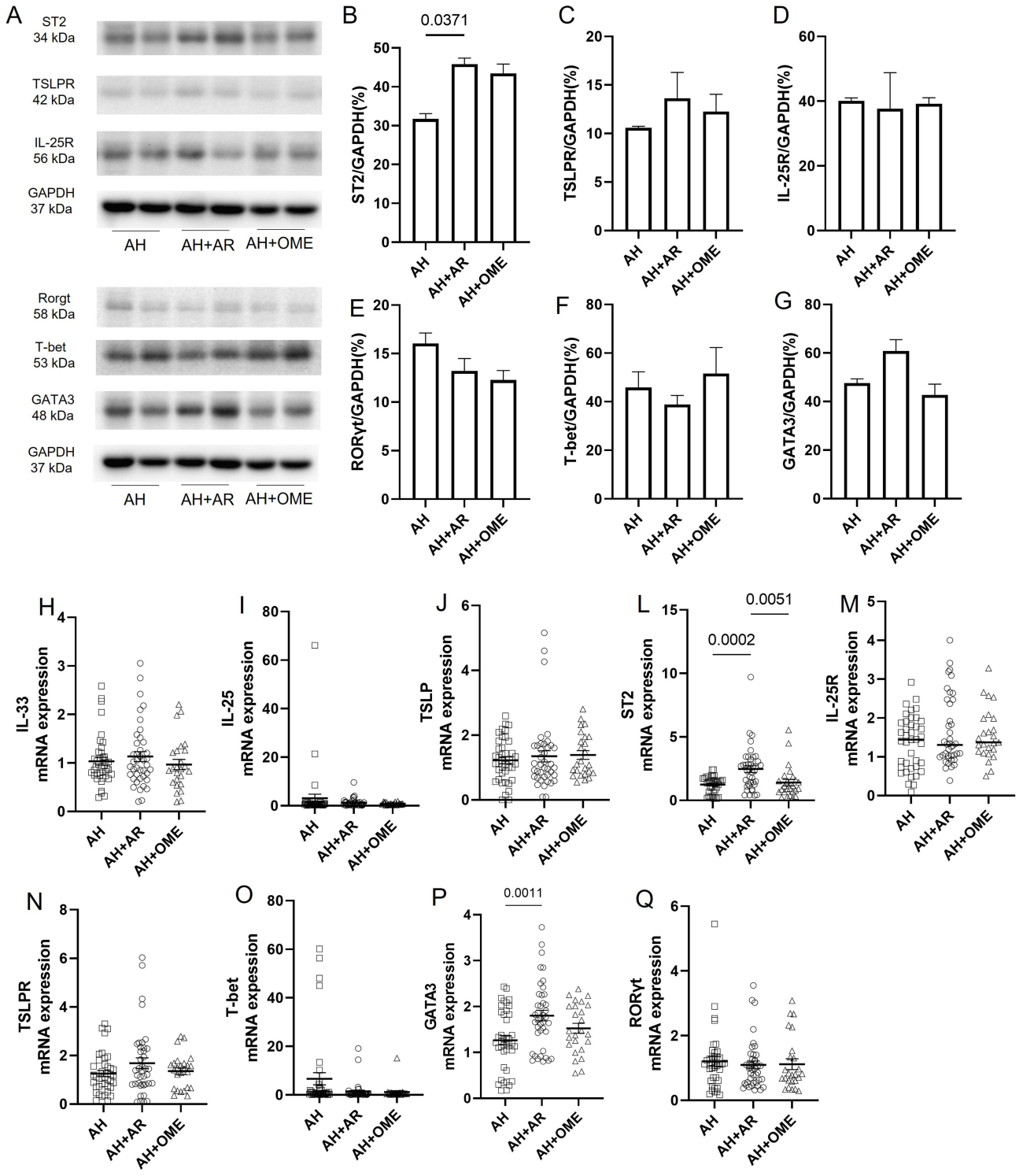

Figure 5 Expression of alarmin receptors and typical transcription factors in adenoid tissue. Western blot (**A-G**) and RT-PCR analyses (**H-Q**) were conducted to assess the expression of IL-33, ST2, and GATA3 in adenoid tissue from AH, AH+AR, and AH+OME groups. The results showed significantly higher levels of IL-33 and ST2 proteins in the AH+AR group compared to the AH and AH+OME groups (**A-B**). ST2 mRNA expression was also significantly elevated in the AH+AR group compared to both the AH ($p = 0.0002$) and AH+OME ($p = 0.0051$) groups (**L**). Conversely, GATA3 protein expression was significantly higher in the AH group compared to the AH+AR group ($p = 0.0011$, **P**). No significant differences were observed in the protein expression of TSLPR, IL-25R, RORγt, T-bet, and GATA3 in adenoid tissues among the AH, AH+AR, and AH+OME groups (**C-G**). Similarly, there are no significant differences in the mRNA expression of IL-33, IL-25, TSLP, IL-25R, TSLPR, T-bet, and RORγt in adenoid tissues among the groups (**H**, **I**, **M-P**, **Q**).

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AR, and the presence of *Streptococcus pneumoniae* in the oropharynx.[34] Recent studies have reported increased levels of IL-4, IL-11, and IFN-gamma, while demonstrating decreased levels of IL-1, TNF-α, and TGF-β in purulent and mucous exudates of the middle ear, pointing towards a prominent role of humoral defense responses in the pathogenesis of OME.[35–37] IL-17A has been shown to be upregulated in adenoid tissues from patients with AH and pneumococcal carriage.[38,39] IL-17 has been identified as a potential therapeutic target in a rodent model of OME. Based on gene expression data implicating IL-17 in otitis media, Zhang et al demonstrated that an IL-17 inhibitor effectively reduced otitis media severity in rats.[40] In our study, we observed higher expression levels of IL-17A in both sera and tissues of the AH+OME group compared to the AH and AH+AR groups, which aligns with previous findings.

Currently, surgical intervention, primarily adenoidectomy, remains the standard treatment for symptomatic AH. However, due to the potential risks associated with surgery, including postoperative complications and the possibility of recurrence, particularly in patients with AH+AR, there is a strong need for less invasive and more effective treatment options.[6,41] Our findings suggest that targeting the IL-33/ST2/ILC2 axis may represent a novel therapeutic strategy for AH+AR. By inhibiting IL-33 signaling, blocking ST2 receptor activation, or modulating ILC2 function, it may be possible to reduce inflammation, attenuate tissue remodeling, and potentially mitigate the need for surgery in selected patients.

AH is predominantly a pediatric condition, with children exhibiting a considerably higher prevalence compared to adults. While less common in adulthood, adult AH can still manifest, with reported prevalence rates in individuals experiencing nasal obstruction varying significantly, ranging from 21% to 63.6% in different cohorts.[42,43] These discrepancies likely reflect variations in study populations and diagnostic methodologies. In adults, AH is frequently linked to chronic inflammation, recurrent infections, or underlying systemic conditions such as HIV or malignancies.[44] The etiology of adult AH is often multifactorial, encompassing the persistence of childhood adenoidal tissue, re-proliferation of previously regressed adenoids in response to infectious or irritant stimuli, and ongoing chronic inflammatory processes.[42] Given the pronounced prevalence in younger age groups, our study specifically focuses on pediatric AH, and all adenoid samples analyzed were obtained from children aged 2 to 12 years undergoing adenoidectomy.

In our study, the gender distribution varied across the different patient groups. The Adenoid Hypertrophy (AH) group comprised 21 males and 19 females (52.5% male). In the AH + Allergic Rhinitis (AR) group, we observed 25 males and 15 females (62.5% male), while the AH + Otitis Media with Effusion (OME) group showed a greater male predominance with 19 males and 6 females (76% male). This higher proportion of male patients in the combined AH + AR and AH + OME cohorts was not a pre-selection criterion but rather reflects the demographic characteristics of the patient population seeking treatment at our clinic during the study period. These observations align with a substantial body of literature reporting a greater incidence of adenoid hypertrophy in males. For instance, studies have consistently noted a male predominance, with reported male percentages in AH cohorts often exceeding 60% (eg, 65.3% male in one study's AH group compared to 49.1% in controls).[45] Another investigation similarly found 71.9% of adenoid hypertrophy patients to be male,[46] and a male-to-female ratio of 1.6:1 has also been reported.[47] The precise reasons for this observed male prevalence are still under investigation and are likely multifactorial. Hypotheses include differential exposure to environmental factors, as males may engage more frequently in outdoor activities, leading to increased exposure to allergens or pollutants which can contribute to chronic inflammation and adenoid growth.[42,48] The reasons for this male prevalence are still being investigated, and it may be due to a combination of factors, including genetic predispositions or differences in immune response. Furthermore, potential biological differences, such as genetic predispositions or variations in immune responses between sexes, may also play a crucial role. Continued research is needed to fully elucidate the underlying mechanisms driving this male predilection in adenoid hypertrophy.

This study has some limitations. The absence of a healthy control group and group with specific targeted treatment to manage ILC2, which preclude direct comparisons of ILC subset frequencies and cytokine expression in adenoid tissues from healthy group and group with specific targeted treatment to manage ILC2. Furthermore, while this study focused on ILC, a more comprehensive understanding of the immunological landscape in AH requires further investigation into the complex interplay between innate and adaptive immune responses. Future studies should include a broader range of immune cells, such as Treg, Th17, and Breg cells, to elucidate the intricate network of cellular interactions that contribute to the pathogenesis of AH.

Conclusions

In summary, this study provides compelling evidence connecting ILC2s to the activation of the IL-33/ST2 signaling pathway in pediatric patients with AH+AR, underscoring this axis as a significant and potentially novel therapeutic target. Our findings highlight the critical role of ILC2s in the pathogenesis of AH+AR and suggest that modulating the IL-33/ST2 axis may offer a new strategy for treating this condition. Further investigations are warranted to elucidate the detailed mechanisms underlying ILC2 activation and function in AH+AR, and to develop targeted therapies that can effectively modulate this pathway, ultimately improving treatment outcomes for pediatric patients with AH+AR.

Ethics Approval and Consent to Participate

Ethical approval for this study was obtained from the Ethics Committee of Shenzhen Longgang Otolaryngology Hospital (ZSSOM 2021-0910). Written informed consent was obtained from the parents or guardians of all participants involved in the study.

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Disclosure

The authors declare no conflicts of interest associated with this research.

References

1. Geiger Z, Gupta N. *Adenoid Hypertrophy*. StatPearls Publishing; 2024.
2. Pereira L, Monyror J, Almeida FT, et al. Prevalence of adenoid hypertrophy: a systematic review and meta-analysis. *Sleep Medicine Rev*. 2018;38:101–112. doi:10.1016/j.smrv.2017.06.001
3. Niedzielski A, Chmielik LP, Mielnik-Niedzielska G, Kasprzyk A, Bogusławska J. Adenoid hypertrophy in children: a narrative review of pathogenesis and clinical relevance. *BMJ Paediatr Open*. 2023;7(1):e001710. doi:10.1136/bmjpo-2022-001710
4. Wang H, Qiao X, Qi S, Zhang X, Li S. Effect of adenoid hypertrophy on the upper airway and craniomaxillofacial region. *Transl Pediatr*. 2021;10(10):2563–2572. doi:10.21037/tp-21-437
5. Naik PP, Lakshanadeve VM, Kurien M, Thomas K. Adenoidectomy: anatomical versus clinical success. *Indian J Otolaryngol Head Neck Surg*. 2023;75(4):3481–3486. doi:10.1007/s12070-023-03992-y
6. Liu Y, Liu T, Li X, et al. Effects of tonsillectomy and adenoidectomy on the immune system. *Heliyon*. 2024;10(12):e32116. doi:10.1016/j.heliyon.2024.e32116
7. Kheirandish L, Goldbart AD, Gozal D. Intranasal steroids and oral leukotriene modifier therapy in residual sleep-disordered breathing after tonsillectomy and adenoidectomy in children. *Pediatrics*. 2006;117(1):e61–66. doi:10.1542/peds.2005-0795
8. Mnatsakanian A, Heil JR, Anatomy SS. *Head and Neck: Adenoids*. StatPearls Publishing LLC.; 2024.
9. Byars SG, Stearns SC, Boomsma JJ. Association of long-term risk of respiratory, allergic, and infectious diseases with removal of adenoids and tonsils in childhood. *JAMA Otolaryngol Head Neck Surg*. 2018;144(7):594–603. doi:10.1001/jamaoto.2018.0614
10. Cyster JG, Allen CDC. B cell responses: cell interaction dynamics and decisions. *Cell*. 2019;177(3):524–540. doi:10.1016/j.cell.2019.03.016
11. Zhu Y, Wang S, Yang Y, et al. Adenoid lymphocyte heterogeneity in pediatric adenoid hypertrophy and obstructive sleep apnea. Original Research. *Front Immunol*. 2023;14:1186258. doi:10.3389/fimmu.2023.1186258
12. Valsecchi C, Tagliacarne SC, Brambilla I, et al. Detection of IL10-producing B cell (B10) in adenoids of atopic children with adenoidal hypertrophy. *Ital J Pediat*. 2018;44(1):30. doi:10.1186/s13052-018-0471-3
13. Sade K, Fishman G, Kivity S, DeRowe A, Langier S. Expression of th17 and treg lymphocyte subsets in hypertrophied adenoids of children and its clinical significance. *Immunol invest*. 2011;40(6):657–666. doi:10.3109/08820139.2011.575426
14. Lu H-F, Zhou Y-C, Luo -D-D, et al. ILC2s: unraveling the innate immune orchestrators in allergic inflammation. *Int Immunopharmacol*. 2024;131:111899. doi:10.1016/j.intimp.2024.111899
15. Franco RA, Rosenfeld RM, Rao M. first place—resident clinical science award 1999Quality of life for children with obstructive sleep apnea. *ENT ORL-H&N*. 2000;123(1):9–16. doi:10.1067/mhn.2000.105254

16. Sarwar S, Rahman M, Ali M, Alam M, Hossain A, Sanyal NP. Correlation of enlarged adenoids with conductive hearing impairment in children under twelve. *Bangladesh J Otorhinolaryngol*. 2016;21:62–68. doi:10.3329/bjo.v21i2.27641
17. Songu M, Islek A, Imre A, et al. Risk factors for otitis media with effusion in children with adenoid hypertrophy. *Acta Otorhinolaryngol Ital*. 2020;40(2):133–137. doi:10.14639/0392-100x-2456
18. Jing Z, McCarron MJ, Dustin ML, Fooksman DR. Germinal center expansion but not plasmablast differentiation is proportional to peptide-MHCII density via CD40-CD40L signaling strength. *Cell Rep*. 2022;39(5):110763. doi:10.1016/j.celrep.2022.110763
19. León B, Lund FE. Compartmentalization of dendritic cell and T-cell interactions in the lymph node: anatomy of T-cell fate decisions. *Immunol Rev*. 2019;289(1):84–100. doi:10.1111/imr.12758
20. Arambula A, Brown JR, Neff L. Anatomy and physiology of the palatine tonsils, adenoids, and lingual tonsils. *World J Otorhinolaryngol Head Neck Surg*. 2021;7(3):155–160. doi:10.1016/j.wjorl.2021.04.003
21. Cho KS, Kim SH, Hong SL, et al. Local atopy in childhood adenotonsillar hypertrophy. *Am J Rhinol Allergy*. 2018;32(3):160–166. doi:10.1177/1945892418765003
22. Drago L, Esposito S, Vecchi ED, et al. Detection of respiratory viruses and atypical bacteria in children's tonsils and adenoids. *J Clin Microbiol*. 2008;46(1):369–370. doi:10.1128/jcm.01819-07
23. Zuliani G, Carron M, Gurrola J, et al. Identification of adenoid biofilms in chronic rhinosinusitis. *Int J Pediatr Otorhinolaryngol*. 2006;70(9):1613–1617. doi:10.1016/j.ijporl.2006.05.002
24. Niu X, Wu ZH, Xiao XY, Chen X. The relationship between adenoid hypertrophy and gastroesophageal reflux disease: a meta-analysis. *Medicine*. 2018;97(41):e12540. doi:10.1097/md.0000000000012540
25. H-l H, Y-q D, Huang H, et al. Inflammatory endotypes of adenoidal hypertrophy based on a cluster analysis of biomarkers. *Int Immunopharmacol*. 2024;127:111318. doi:10.1016/j.intimp.2023.111318
26. Ni K, Zhao L, Wu J, Chen W, HongyaYang L, Li X. Th17/Treg balance in children with obstructive sleep apnea syndrome and the relationship with allergic rhinitis. *Int J Pediatr Otorhinolaryngol*. 2015;79(9):1448–1454. doi:10.1016/j.ijporl.2015.06.026
27. Sade K, Fishman G, Kivity S, DeRowe A, Langier S. Expression of Th17 and Treg lymphocyte subsets in hypertrophied adenoids of children and its clinical significance. *Immunol Invest*. 2011;40(6):524–540. doi:10.1016/j.cell.2019.03.016
28. Shen H, Lin Z, Yuan J, et al. Low vitamin D levels and frequencies of regulatory T cells (Tregs) are associated with adenotonsillar hypertrophy in children. *Int J Pediatr Otorhinolaryngol*. 2020;138:110231. doi:10.1016/j.ijporl.2020.110231
29. Jutel M, Agache I, Zemelka-Wiacek M, et al. Nomenclature of allergic diseases and hypersensitivity reactions: adapted to modern needs: an EAACI position paper. *Allergy*. 2023;78(11):2851–2874. doi:10.1111/all.15889
30. Cianferoni A. Non-IgE-mediated anaphylaxis. *J Allergy clin Immunol*. 2021;147(4):1123–1131. doi:10.1016/j.jaci.2021.02.012
31. Alvarez-Arango S, Kumar M, Chow TG, Sabato V. Non-IgE-mediated immediate drug-induced hypersensitivity reactions. *J Allergy Clin Immunol Pract*. 2024;12(5):1109–1119. doi:10.1016/j.jaip.2024.02.019
32. Hu L, He W, Li J, Miao Y, Liang H, Li Y. The role of adenoid immune phenotype in polysensitized children with allergic rhinitis and adenoid hypertrophy. *Pediatr Allergy Immunol*. 2024;35(6):e14166. doi:10.1111/pai.14166
33. Huo Z, Shi J, Shu Y, Xiang M, Lu J, Wu H. The relationship between allergic status and adenotonsillar regrowth: a retrospective research on children after adenotonsillectomy. *Sci Rep*. 2017;7(1):46615. doi:10.1038/srep46615
34. Chen W, Yin G, Chen Y, et al. Analysis of factors that influence the occurrence of otitis media with effusion in pediatric patients with adenoid hypertrophy. *Original Res Front Pediatr*. 2023;11. doi:10.3389/fped.2023.1098067
35. Saylam G, Ec T, Tatar I, Ozdek A, Korkmaz H. Association of adenoid surface biofilm formation and chronic otitis media with effusion. *Arch Otolaryngol Head Neck Surg*. 2010;136(6):550–555. doi:10.1001/archoto.2010.70
36. Cassano P, Cassano M. Otitis media and immunological alterations of Waldeyer's ring. *Adv Otorhinolaryngol*. 2011;72:164–169. doi:10.1159/000324783
37. Bernstein JM. Immunologic reactivity in the middle ear in otitis media with effusion. *Otolaryngol Clin North Am*. 1991;24(4):845–858. doi:10.1016/S0030-6665(20)31095-1
38. Huang CC, Wu PW, Chen CL, et al. IL-17A expression in the adenoid tissue from children with sleep disordered breathing and its association with pneumococcal carriage. *Sci Rep*. 2018;8(1):16770. doi:10.1038/s41598-018-35169-x
39. Kwon OE, Park SH, Kim SS, et al. Increased IL-17 and 22 mRNA expression in pediatric patients with otitis media with effusion. *Int J Pediatr Otorhinolaryngol*. 2016;90:188–192. doi:10.1016/j.ijporl.2016.09.025
40. Zhang N, Qian T, Sun S, et al. IL-17 is a potential therapeutic target in a rodent model of otitis media with effusion. *J Inflamm Res*. 2022;15:635–648. doi:10.2147/jir.S338598
41. Tamir SO, Schwarz Y, Hazan I, Cohen Michael O, Tshori S, Marom T. Medical treatment does not reduce surgery rates in children with adenoid hypertrophy. *Int J Pediatr Otorhinolaryngol*. 2024;176:111836. doi:10.1016/j.ijporl.2023.111836
42. Rout MR, Mohanty D, Vijaylaxmi Y, Bobba K, Metta C. Adenoid hypertrophy in adults: a case series. *Indian J Otolaryngol Head Neck Surg*. 2013;65(3):269–274. doi:10.1007/s12070-012-0549-y
43. Kothekar A, Vallur S. Adenoid hypertrophy and endoscopic adenoidectomy in adults: our experience. *Indian J Otolaryngol Head Neck Surg*. 2022;74(2):1067–1072. doi:10.1007/s12070-020-02142-y
44. Geiger Z, Gupta N. *Adenoid Hypertrophy*. Treasure Island (FL): StatPearls Publishing; 2023.
45. Avital A, Chaim S. Adenoid hypertrophy in young children is associated with increased respiratory morbidity and might be connected to supine feeding position. *Arch Pediatr Dev Pathol*. 2020;6(1):1025.
46. Bhise S, Hippargekar P, Kothule S, Dhanwe M, Ngullie O. A clinical study of persistent adenoid and its secondary effect. *Indian J Otolaryngol Head Neck Surg*. 2023;75(4):3507–3513. doi:10.1007/s12070-023-03841-y
47. Alam M, Razzak MA, Rahman SMA, Islam S, Akter S. Evaluation of risk factors and clinical presentation of adenoid hypertrophy among children attending the pediatric outpatient department of tertiary care hospital in Dhaka division. *Int J Contemp Pediatr*. 2022;9(12):1136–1140. doi:10.18203/2349-3291.ijcp20223058
48. Kim S-W, Taranto-Montemurro L. When do gender differences begin in obstructive sleep apnea patients? *J Thoracic Dis*. 2019;11:S1147–S1149. doi:10.21037/jtd.2019.04.37