**The role of adenoid immune phenotype in polysensitized children with allergic rhinitis and adenoid hypertrophy**

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**Abstract**

**Background:** There has been increasing interest in elucidating the relationship between adenoid hypertrophy (AH) and allergic rhinitis (AR). However, the impact of aeroallergen sensitization patterns on children concurrently experiencing AH and AR remains unclear.

**Methods:** Patients aged 2-8 years **(**January 2019 to December 2022) with nasal symptoms were assessed for allergies, adenoid size and respiratory viral infection history. The levels of serum total immunoglobulin E (IgE) and specific IgE and flexible nasal endoscopy were performed. We analyzed the relationship between AH and sensitization patterns and lymphocyte subpopulations in adenoid samples using flow cytometry.

**Results:** 5281 children were enrolled in our cohort. 56.5% of children was diagnosed with AR and 48.6% with AH. AR was more prevalent in AH children compared to nAR. Compared to non-sensitized, those with AR polysensitized to molds had a higher prevalence of AH (adjusted OR 1.61, 95%CI 1.32-1.96) and a greater occurrence of two or more respiratory viral infections, particularly in cases with adenoidectomy. In AH-AR children, adenoid tissues showed reduced frequencies and corrected absolute counts of regulatory T cells (Tregs), activated Tregs, class-switched memory B cells (CSMB), natural killer (NK) T cells and NK subpopulations compared to AH-nAR children. Polysensitization in AH-AR children correlated with lower CSMB frequencies.

**Conclusion:** Polysensitivity to molds significantly increased the risk of AH in children with AR. Adenoids of AR children demonstrated less number of B cells, NK cells and Treg cells with an effector/memory phenotype, which was closely linked to sensitization models and respiratory viral infection, particularly concerning CSMB.

**Keywords** adenoid hypertrophy, allergy, children, flow cytometry, immune monitoring

**Key Message**

There has been increasing interest in identifying the relationship between adenoid hypertrophy and allergic rhinitis. However, the impact of aeroallergen sensitization patterns on children concurrently experiencing adenoid hypertrophy and allergic rhinitis remains unclear. In our study, we explored the immunological mechanisms that underlie these sensitization patterns in the context of adenoid hypertrophy and allergic rhinitis.

Our results indicate that in children aged 2-8 years in Shanghai who have allergic rhinitis, those with **polysensitivity to molds are at an increased risk of developing adenoid hypertrophy.** Furthermore, **among children with adenoid hypertrophy, those who were polysensitized to molds were more susceptible to respiratory viral infections**. **In adenoid tissues of children with adenoid hypertrophy and allergic rhinitis, B cells, NK cells and Treg cells with an effector/memory phenotype demonstrated lower frequencies and counts. Additionally, our study reveals reduced frequencies of memory B cells and class-switched memory B cells in children with adenoid hypertrophy and polysensitization to molds**, which is closely linked to sensitization models and a heightened susceptibility to respiratory viral infection, particularly concerning class-switched memory B cells. To our knowledge, **this is the first study to compare the immune phenotype in adenoid tissue between polysensitization and monosensitization in allergic children with adenoid hypertrophy, focusing on regular immune cells and T/B/NK cells subpopulation parameters.** Further research may hold the potential to enhance both diagnostic and therapeutic approaches for this condition.

**1 INTRODUCTION**

Allergic rhinitis (AR) is defined clinically by nasal hypersensitivity symptoms induced by an immunoglobulin E (IgE)-mediated inflammation after the exposure of the nasal mucous membranes to an offending allergen.1 As a critical public health, medical and economic problem, AR affects 2%-25% in children.1,2 Adenoid hypertrophy (AH) is a common comorbidity in pediatric AR with a frequency of 12%-21.2%.3,4 As a peripheral lymphoid organ, adenoid tissue locates in the roof of the rhinopharynx and contributes to the development of immunity against inhaled microorganisms in early life. Enlarged adenoid can cause mechanical blockage and act as a reservoir for pathogens, contributing to recurrent rhinosinusitis and upper respiratory infections.5

Aeroallergen constitutes established risk factors in the onset and exacerbation of AR.1 Notably, in central China, the predominant indoor allergenic source is the house dust mite (HDM).6 The prevalence of indoor dampness and mold is widespread in China, and these conditions have been identified as possessing the robust correlation with the development of asthma and AR.7 In addition, polysensitization has been well studied in several research as it may be associated with a more impaired quality of life than monosensitization in children.8 Recent research has reported discrepancies in the inflammatory and clinical patterns depending on the specific causal allergen, indicating variations in the immune response.9 However, allergies, recurrent or chronic infections trigger an inflammatory response that can cause the adenoids to become enlarge.10 Another case report by Sui H et al. reported a 10-year-old boy with AH, severe allergic asthma, and AR has been significantly improved on adenoid size after the four weeks of treatment with omalizumab, a monoclonal antibody used for allergic asthma.11

Our study aims to characterize the sensitization profiles to inhaled allergens in children with nasal complaints in urban China. Additionally, we evaluate the relationship between presence of AH and aeroallergen sensitization patterns in children with AR. Furthermore, we also investigate the immune phenotype in allergic adenoid to gain a better understanding of the underlying immunological mechanisms related to AH and allergy.

**2 METHODS**

**2.1 Study Design and Participants**

The observational prospective cohort study involving children aged 2-8 years was conducted at Shanghai Children’s Medical Center, a large urban tertiary teaching hospital in Shanghai, China. Patients who visited the Ear, Nose and Throat clinic due to nasal obstruction and/or snoring lasting more than 4 weeks for suspicion of either having allergies or enlarged adenoid were enrolled between January, 2019 and December, 2022. Patients’ age, gender, clinical symptoms were recorded. Moreover, the history of respiratory viral infection was obtained for children who underwent adenoidectomy. Serum total IgE (tIgE) and specific IgE (sIgE) levels and flexible nasal endoscopy were performed in all patients. AR diagnosis was based on Allergic Rhinitis and its Impact on Asthma (ARIA) guideline.1 Children with autoimmune diseases, craniofacial deformity, abnormal of anatomic structure of nasal cavity, congenital malformation syndromes, such as Down’s syndrome, mucopolysaccharidosis syndrome, hemifacial microsomia, or those using nasal steroids, antihistamines, or antileukotrienes within 4 weeks prior to the evaluation were excluded. The flow chat was shown in Figure 1. According to the distribution pattern of airborne pollen grains in Shanghai, visiting time of the patients were divided into the seasons of grains distribution peak including March, April and September and the seasons of remaining months.12 The study was approved by the ethics committee of Shanghai Children’s Medical Center (SCMCIRB-Y2020100) and adhered to the principles of Helsinki Declaration. All parents or guardians signed an informed consent form.

**2.2 Evaluation of adenoid hypertrophy presence and determination of sensitization**

AH was considered based on history and endoscopic evaluation. The adenoids were graded depending on volume of adenoid to the size of the nasopharyngeal airway. Adenoids occupying more than 50% of the nasopharynx were referred to AH.13

Serum was collected and analyzed by ImmunoCAP (Phadia, Uppsala, Sweden) for tIgE and sIgE antibodies to the following allergens: HDM (Dermatophagoides pteronyssinus and Dermatophagoides farinea), molds (Penicillium chrysogenum, Cladosporium herbarum, Aspergillus fumigatus, and Alternaria alternata), cat dander and dog dander, cockroaches (Blatella germanica), a mixture of tree pollens (alder, hazel, poplar, elm, and willow), and a mixture of grass pollens (common rageweed, mugwort, Chrysanthemum leucanthemum, dandelion, and Solidago decurrens). Quantified tIgE levels and allergen-specific IgE levels were recorded. According to the manufacturer’s instructions, a serum tIgE level ≥60 kU/L was defined as elevated. A serum sIgE level ≥0.35 kUA/L was defined as sensitization and ≥50 kUA/L was classified as a high level.

Sensitization to one or more allergens was categorized as monosensitization and polysensitization respectively. Specifically, polysensitized to molds/HDM was characterized by the presence of molds/HDM-specific IgE in conjunction with other allergen-specific IgE.

**2.3 Adenoidal single-cell collection and processing analysis**

Children underwent adenoidectomy and met any of the following criteria was excluded from adenoidal single-cell collection processing: usage of intranasal steroid spray, antihistamines, or antileukotrienes within 4 weeks before surgery, presence of acute or chronic respiratory disease within 4 weeks before surgery, immunosuppression caused by any condition, and diagnosis of congenital heart disease or genetic disorder.

2\*2 millimeters adenoid samples were collected in a vacuum tube (BD Biosciences, USA) containing 2 ml of phosphate-buffered saline (PBS). All samples were tested immediately or stored at 4℃ for no more than 2 hours after collection. Mince the tissue into small pieces using a scalpel or scissors to dissociate the cells. Filter the adenoid cells through a cell strainer to remove any remaining tissue debris and obtain a single-cell suspension. Count the cells using a hemocytometer or automated cell counter, and adjust the concentration to the desired level for downstream applications. Use fluorescence-activated cell sorting (FACS) or microfluidics to isolate specific cell populations based on their physical and/or molecular characteristics. Single-cell suspension was then analyzed by flow cytometry.

**2.4 Regular immune status and T/B/natural killer/monocyte cell subpopulation panel in flow cytometry**

Single-cell suspension was then incubated with DuraClone antibody cocktails (Immune Monitoring, Beckman Coulter Life Sciences, Bangalore, India) to stain immune cells in adenoid. The following immune cells were identified: basic, T cell, B cell, cell identification, and activation marker cell surface expression. The specific staining protocols are included in Table S1.

Flow cytometric fluorescent anti-human monoclonal cell surface antibody (dry powder) tubes directed to T cell, B cell, and natural killer (NK) cells (DuraClone IM) were purchased from Beckman Coulter (Bangalore, India). The details of every fluorochrome-conjugated antibody, the schemes of every fluorochrome channel and the compensation controls (each of a single color) are presented in Table S2-5. Briefly, 300μL of each sample of single-cell suspension was washed twice using 1x PBS to remove free immunoglobulins. 100μL of single-cell suspension was stained with fluorescent antibodies for 15 min in the dark (room temperature). The erythrocytes were removed by adding one mL of FACSTM Lysing Solution (BD Biosciences, USA) and incubated for 10 min in the dark (room temperature). Then, the cells were rinsed twice and resuspended in staining buffer (PBS containing 2% fetal bovine serum) prior to acquisition. Every tube was stopped for testing when 30000 cells were collected.

**2.5 T helper cells & regulatory T cells subpopulation test panel**

CD3+CD4+ T cells were stained with fluorescent-labeled monoclonal antibodies directed against human CD3-APC-A750, CD4-APC, CD25-PC5.5-A, CD127-PE, CD45RA-ECD, and HLDA-DR-PB (both from Beckman Coulter) for 15 min. CD4+CD25highCD127lowCD45RA+ naive regulatory T cells (Tregs), CD4+CD25highCD127lowCD45RA− memory Tregs, and CD4+CD25highCD127lowHLA-DR+ activated Tregs subsets were isolated.

Cells were also fluorescently stained with CD183/CXCR3-FITC, CD196/CCR6-PC7 (both from Beckman Coulter). CD4+ T helper (Th) cells were identified by excluding CD4+CD25highCD127low Treg cells. The CD4+ Th cells were further analyzed for CD183 and CD196 expression. Th2 (CD4+CD25low/negCD127highCD183-CD196-), Th1 (CD4+CD25low/negCD127highCD183+CD196-) cells, Th17 (CD4+CD25low/negCD127highCD183-CD196+) cells, and Th1likeTh17 (CD4+CD25low/negCD127highCD183+CD196+) cells could then be distinguished.

**2.6 Data analysis**

A 11-Color DxFlex Flow Cytometer (Beckman Coulter, Brea, CA, USA) was used to analyze the samples. The CytoFlex software (verison 2.00.283; Beckman Coulter, Brea, CA, USA) was carried out to investigate the collected flow cytometric information. Setup, calibration and quality control procedures(QC) were conducted according to the manufacturer’s instructions. In brief, to set up the DxFLEX instrument, Daily QC beads (Beckman Coulter) were used. For compensation, single color-stained VersaComp antibody capturing beads (Beckman Coulter) were used and an automatic compensation was performed according to the manufacturer’s instructions. The adhesive doublets were removed by two forward scatter parameters (width vs. height). We used CD45 and side scatter to gate leukocytes.

The gating strategies for regular immune status (CD3+CD4+ T cells, CD3+CD8+ T cells, NK cells, and CD19+ B cells), T cell subpopulation, B cell subpopulation, Th cell and Treg subpopulations are presented in Figure S1-4. Details are described in supplementary material.

To calculate the absolute numbers (cells/μL) of lymphocytes subpopulation for accurate in the tissue samples, corrected absolute counts (c-counts) of cells are calculated by The CytoFlex software using the following formula: corrected cell population absolute count test volume =10000\* (events in cell population/events in absolute count bead region) \* (beads of each test/test volume) / the absolute number of lymphocytes.

**2.7 Statistical analysis**

Data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM Corp, Armonk, NY, USA). Continuous variables were presented either as mean ± standard deviation (SD) or as median (interquartile range, IQR), depending on the normality of distribution. Categorical variables were expressed as frequencies and percentages. Student’s *t* test was used for the comparison of normal and homogeneous distribution of the parametric values. Chi-square and Mann–Whitney *U* test were used to compare nonparametric values. Binary logistic regression analysis was applied to estimate odds ratio (OR) and 95% confidence intervals (CIs) for examining the association between aeroallergen sensitizations and AH, while controlling for potential confounding factors such as age, gender and seasons. A *p* value below 0.05 was considered statistically significant. GraphPad Prism 6.04 (GraphPad Software, Inc., La Jolla, CA, USA) was used for graphical representation.

**3 RESULTS**

**3.1 Study population**

5281 subjects aged 2-8 years were enrolled in our cohort from January, 2019 to December, 2022. Among these patients, 3192 (60.4%) were boys and 2089 (39.6%) were girls. The mean age of the participants was 4.94±1.60 years old. In our study, 2984 (56.5%) children were diagnosed with AR, with a higher prevalence in boys compared to girls (58.9% vs. 52.9%, *p* < 0.001). AR children were significantly older than non-AR (nAR) children (5.04±1.61 vs. 4.80±1.59, *p* < 0.001).

AH was detected in 2567 (48.6%) children, with 1771 having AR and 796 having nAR. The prevalence of AH was significantly higher in AR children compared to nAR children (59.3% vs 34.7%, *p* < 0.001). There were no gender differences between children with and without AH. However, among children with AR, younger ones were more were more likely to experience AH than older ones (4.81±1.54 vs. 5.39±1.64, *p* < 0.001).

Furthermore, the children with non-AH (nAH) had significantly higher tIgE levels compared to children with AH (163.00kU/L [IQR: 55.05-389.00] vs. 102.00kU/L [IQR:21.50-267.00], *p* < 0.001). Detailed descriptive statistics of the included children was presented in Table 1.

Among children with AR, HDM accounted for the highest prevalence at 76.8%, followed by molds (30.4%) and animal dander (22.9%). The profiles of sensitization to aeroallergens in children with AR and AH was shown in Figure 2 and described in detail in supplementary material.

**The associations between sensitization patterns and the presence of AH**

The associations between presence of AH and sensitization patterns of the top three allergens (HDM, molds, and animal dander) in AR children was evaluated. A total of 2984 children were divided into three groups based on their sensitization patterns: 573 (19.2%) children were polysensitized to molds, 334 (11.2%) were monosensitized to molds, and 2077 (69.6%) were non-sensitized to molds. Among children polysensitized to molds, HDM (86.0%) was the most common additional allergen in children followed by cat dander and dog dander (37.9%).

The prevalence of AH was significantly higher among AR children who were polysensitized to molds compared to those who were monosensitized or non-sensitized (*p* < 0.001) (Table 2). Furthermore, AR children who were polysensitized to molds were more likely to have elevated tIgE level (*p* < 0.001) and high sIgE level (*p* = 0.019) compared to the other groups. After considering the potential confounding facts such as age, gender, and seasons, binary regression analyses suggested that polysensitivity to molds increased the risk of AH in children with AR aged 2-8 years (adjusted OR 1.61, 95%CI 1.32 to 1.96, *p* < 0.001). However, no significant association was observed between presence of AH and sensitization patterns to HDM and animal dander in AR children.

**Sensitization patterns in children with the history of respiratory viral infection undergoing adenoidectomy.**

When reviewing medical history of children who underwent adenoidectomy during the study period, we identified a subgroup of them (n=80), including 35 nAR children, 24 who were monosensitized to HDM, 8 who were monosensitized to molds, and 13 who were sensitized to HDM and molds. Among this subgroup, serum immunoglobulin G (IgG) antibodies to Epstein-Barr virus (EBV), cytomegalo virus (CMV), respiratory syncytial virus (RSV) and adenovirus (ADV) were detected in the past three months. We observed that the positive rate of past infection history involving two or more viruses was significantly higher in children with polysensitization (84.6%) compared to the other groups (nAR: 40.0%; monosensitized to HDM: 54.2%; monosensitized to molds: 50.0%; *p* = 0.049). This finding suggested that viral infections may play a role in children with AH and various sensitization patterns.

**Regular immune status in adenoid tissues of children with or without AR**

Among the 36 adenoid tissues (26 AH-AR group and 10 AH-nAR group), AH-AR group was characterized by significantly lower cell percentage and c-counts of CD3+CD56+ natural killer T cells(NKTs) compared to the AH-nAR group (0.43±0.44% vs. 0.94±0.37%, *p* < 0.001; 15.60±22.07/μL vs. 40.54±22.52/μL, *p* < 0.001, respectively). Similar trends were observed in the frequencies and c-counts of CD16+CD56high NKs (CD56high NKs) (2.51±4.28% vs. 4.10±3.42%, *p* = 0.037; 5.93±9.24/μL vs. 10.81±7.19/μL, *p* = 0.018), CD16+CD56+ NKs (2.35±1.73% vs. 4.61±3.36%, *p* = 0.042; 6.28±4.74/μL vs.12.08±7.18/μL, *p* = 0.010), and HLA-DR+ NKs (20.39±10.24% vs. 37.88±19.56%, *p* = 0.009; 6.68±6.27/μL vs. 17.77±9.64/μL, *p* < 0.001). A reduction in the percentage (20.01±15.08% vs. 42.90±15.61%, *p* = 0.001) and c-counts (731.20±751.44/μL vs. 1866.87±940.36/μL, *p* = 0.001) of HLA-DR+CD3+ cells was observed in AH-AR group. Detailed information is presented in Figure 3a-d and representative flow cytometry plots of adenoid tissues inAH-AR and AH-nAR children are shown in Figure S5a-d. However, no significant difference was found in the cell percentages and c-counts of CD3+CD4+ T cells, CD3+CD8+ T cells, and CD14+ monocytes from leukocytes and subgroups (CD14highCD16-monocytes, CD14highCD16+monocytes and CD14lowCD16high monocytes) between AH-AR and AH-nAR.

**Distribution and counts of T/B cell subpopulations in adenoid tissues of children with or without AR**

Compared to AH-nAR group, AH-AR groups had the higher percentages and c-counts of both CD19+ B cells (64.54±10.41% vs. 55.90±9.60%, *p* = 0.025; 6454.41±1040.57/μL vs. 5589.62±960.25/μL, *p* = 0.024) and IgD+CD27- naive B cells (63.79±8.77% vs. 55.53±8.72%, *p* = 0.041; 4131.50±955.18/μL vs. 3120.80±812.95/μL, *p* = 0.012). Conversely, a marked reduction in the percentage of memory B cells (31.17±7.84% vs. 41.96±8.66%, *p* = 0.003) and in the percentage and c-counts of IgM-IgD-CD27+CD38low/neg class-switched memory B (CSMB) cells (21.63±8.16% vs. 32.22±7.68%, *p* = 0.003; 1369.11±553.69/μL vs. 1798.23±484.02/μL, *p* = 0.031) were founded in AH-AR children compared to AH-nAR group. Detailed information is presented in Figure 3e-h and representative flow cytometry plots are shown in Figure S5e-h. The percentages and c-counts of T cells subpopulation were comparable in two groups and did not differ significantly.

**Distribution and counts of Treg cell subpopulations in adenoid tissues of children with or without AR**

In the AH-AR group, the percentage and c-counts of total Treg (CD4+CD25highCD127low) was significantly lower than that of AH-nAR group (16.68±6.72% vs. 21.67±3.89%, *p* = 0.005; 426.99±184.97/μL vs. 693.47±190.14/μL, *p* = 0.001). And we also observed a significant reduction in percentage (29.28±24.35% vs. 43.23±14.27%, *p* = 0.016) and c-counts (123.70±127.40/μL vs. 304.58±134.64/μL, *p* < 0.001) of activated Tregs (CD4+CD25highCD127lowHLA-DR+) in AR-AH children. Detailed information is presented in Figure 3i-j. Representative flow cytometry plots of adenoid tissues in AH-AR and AH-nAR children are shown in Supplementary Figure S5i-j. There was no significant difference in the percentages and c-counts of memory Treg (CD4+CD25highCD127lowCD45RA-), naive Treg (CD4+CD25highCD127lowCD45RA+) and Th subpopulation (CD4+CD25low/negCD127highCD183-CD196+ Th17, CD4+CD25low/negCD127highCD183-CD196- Th2, CD4+CD25low/negCD127highCD183+CD196- Th1, and CD4+CD25low/negCD127highCD183+CD196+ Th1likeTh17).

**Immunologic phenotyping in adenoid tissues of monosensitized and polysensitized to molds**

For further investigation, we analyzed the frequencies and c-counts of lymphocyte populations of children with AH who were polysensitized to molds and HDM (AH-Molds+HDM+, n=9) and monosensitized to HDM (AH-HDM+, n=17). Ones polysensitized to molds and HDM had a significant higher in percentages and c-counts of both CD19+ B cells (70.93±4.22% vs. 61.17±11.19%, *p* = 0.033; 7092.46±421.69/μL vs. 6116.62±1119.24/μL, *p* = 0.033) and IgD+CD27- naive B cells (70.46±6.43% vs. 60.26±7.82%, *p* = 0.005; 4994.58±504.78/μL vs. 3674.58±810.77 /μL, *p* < 0.001) in the adenoid tissues compared to children monosensitized to HDM. In contrast, the frequency of memory B cells (24.80±5.69% vs. 34.54±6.70%, *p* = 0.002) and IgM-IgD-CD27+CD38low/neg CSMB cells (15.80±5.69% vs. 24.72±7.66%, *p* = 0.003) were significantly lower in children polysensitized to molds and HDM compared to ones monosensitized to HDM. Detailed information is presented in Figure 4.

**4 DISCUSSION**

In this study, we investigated how children with AH in urban China react to allergens and the types of allergies they commonly experience. Furthermore, we explored how the immune phenotype in adenoid tissue responds to different patterns of allergen sensitization. Our main findings are that lower Treg and active Treg levels in allergic adenoid tissue, decreased levels of B memory and CSMB cells, and fewer of subsets of NK cells, particularly in children with aeroallergen polysensitization. As far as we know, this is the first study that compares the immune phenotype in adenoid tissue between polysensitization and monosensitization in allergic children with AH, focusing on regular immune cells and T/B/NK cells subpopulation parameters.

According to previous report, AH is most prevalent in children aged 4-7 years.14 In our study, the mean age of AR children suffering from AH was 4.81±1.54. AR children at younger ages were more likely to experience AH. Previous studies have also reported a higher occurrence of AH in allergic conditions.4,15,16 In our study, AH occurred significantly more frequently in AR children. We identified a unique sensitization profile of AR-AH children in urban China, revealing that HDM was the most common aeroallergen in these cases. However, the prevalence of AH in children sensitized to HDM was significantly lower than in those non-sensitized to HDM. This result is consistent with previous studies4,15-17 and suggests that HDM may not be the main contributing aeroallergen for AH in children with AR. On the other hand, sensitivity to molds was found to be associated with AH in children with AR.15,17,18 Atan Sahin O et al. reported a significantly higher prevalence of AH in children exposed to molds.18 In our study, AR children with AH exhibited a significantly higher prevalence of sensitivity to molds.

Additionally, a significant correlation was observed between the presence of AH and the aeroallergen sensitization pattern of polysensitivity to molds in children with AR. Our study’s results suggest that being polysensitized to molds increases the risk of AH in children with AR aged 2-8 years by more than sixty percent. Obviously, AH should be particularly considered and investigated in children who are polysensitized to molds. Monosensitized and polysensitized patients exhibit distinct immune response. Polysensitized children, in comparison to monosensitized ones, have higher levels of total IgE and allergen-specific IgE, as reported in our findings. There is difference in the IgE immune response between monosensitized and polysensitized patients, which suggests a clear division between low and high IgE response. This finding raises the possibility that polysensitized individuals may be more prone to developing AH.

To better understand the intrinsic characteristics that contribute to susceptibility to AH in monosensitized children and polysensitized children, we conducted an extensive comparison immunological phenotyping in adenoid tissue between AH-AR and AH-nAR group. Specifically, we compared children who were polysensitized to molds and HDM, with who were monosensitized to HDM. In adenoid tissue, we noticed a significant reduction in the percentages and c-counts of NKTs, CD56high NKs, CD16+CD56+ NKs, and HLA-DR+ NKs in AH-AR children compared to AH-nAR ones. However, no such discrepancy was observed when comparing polysensitized and monosensitized cases. Although the role of NK cells in patients with allergic disease is not yet fully understood, but recent studies suggest their involvement in allergies.19,20 These studies have shown reduced frequencies of CD16+CD56+ NKs in polyallergic patients.19 The significance of NK cells in innate immunity and their contribution to immune responses, along with their distinct cytokine patterns, might be crucial in modifying the cytokine milieu and the induction of T cell deviation and IgE production.21,22 It is well known that NK cells appear to play a crucial role in viral infection.21,23 The adenoids, which local virus colonization, can become enlarged as a consequence of their inflammatory response, particularly due to viral infection. These results suggest that children with AH and AR may contribute to the delayed clearance or persistence of virus in children.

The current model of B cell memory suggests that memory B cells play a vital role in defending against variant pathogens.24 For many infectious diseases, immunological memory is acquired after a single infection and this process relies largely on the acquisition memory B cells. These memory B cells have the ability to produce high affinity, antigen-specific antibodies and respond effectively to pathogens and their variants upon reinfection.25,26 Class switching to different immunoglobulin isotypes is regulated by B cell activation and cytokines. B cells play a significant and major well-established role in allergies by producing IgE antibodies that specifically target components of allergens. However, B cells may also have protective functions in allergy. For instance, they can produce IgG antibodies, which can provide a protective response against allergens. Additionally, B cells may act as regulatory B cells, helping to regulate the immune system and maintain a balanced response to allergens. In our study, we observed that AH-AR children had a higher frequency and c-counts of B cells and naive B cells compared to AH-nAR children. However, AH-AR children showed a lower frequency and c-counts of CSMB cells. Furthermore, the frequencies of memory B cells and CSMB cells were significantly reduced in children who were polysensitized to molds and HDM compared to those who were monosensitized to HDM. These results indicate that AH-AR children and those polysensitized to molds and HDM may have a weaker defense mechanism when challenged by pathogens, which was consistent with findings in our study that children polysensitized to molds and HDM were more likely to experience respiratory viral infections. The presence of AH and the specific aeroallergen sensitization pattern in AR children may be associated with an increased susceptibility to infection.

Genetic and immunological evidence strongly supports the crucial role of Treg cells in fostering tolerance to allergens and preventing allergic disorder. These cells play a vital role in suppressing allergic and inflammatory reactions, as well as responding to infections and tumors. Our study revealed lower levels of Treg and activated Treg cells in AH-AR children compared to AH-nAR children, although these findings were not evident in various sensitization models. The presence of Treg cells in adenoid tissue could play a part in the persistence of pneumococcus in children.27 Treg cells help limit immune responses to microbial infections, this may have a dual effect, preventing inflammation-related local tissue damage and autoimmunity, while also potentially contributing to chronicity of infection, particularly for allergic tissue with low Treg counts.

**5 CONCLUSION**

Among children aged 2-8 years with AR, having polysensitivity to molds significantly increased the risk of developing AH. Notably, adenoids of AR children displayed less frequencies and numbers of B cells, NK cells and Treg cells with an effector/memory phenotype, which was closely linked to sensitization models and respiratory viral infection, particularly in the case of CSMB cells. Conducting further studies to investigate the specific virus components and the underlying mechanisms involved in sensitization models that lead to the attenuation of memory B cells in adenoid tissues could have significant implications, ultimately leading to improve the management of AH in children.

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**Data availability**

Datasets generated and/or analyzed during the current study will be accessible from the corresponding author on reasonable request.

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**FIGURE 1** The flow chart of this study. tIgE, serum total IgE; sIgE, serum specific IgE; AR, allergic rhinitis; nAR, non-allergic rhinitis; AH, adenoid hypertrophy; nAH, non-adenoid hypertrophy.

**fIGURE 2** Profiles of aeroallergens sensitization in children with AR and AH. **a** The prevalence of sensitization to aeroallergens increased with age. **b** The sensitization profile in males and females.Boys with AR showed significantly higher sensitivity to HDM and a mixture of grass pollens compared to girls. **c** The sensitization profile in AR-AH and AR-nAH children. AR children with AH exhibited a significantly higher prevalence of sensitivity to molds and a mixture of tree pollens, while sensitivity to HDM was more common among children with nAH. \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001.

**fIGURE 3** The counts of lymphocyte subpopulations in adenoid samples between AH-AR (n=26) and AH-nAR (n=10) children. **a** NKTs; **b** CD56high NKs; **c** CD16+CD56+ NKs; **d** HLA-DR+ NKs; **e** B cells; **f** Naive B cells; **g** Memory B cells; **h** CSMB cells; **i** Tregs; **j** Activated Tregs. Midlines indicate the median values. Statistical significances were determined using unpaired, two-tailed Mann-Whitney *U* test. NKTs, natural killer T cells; NKs, natural killer cells; CSMB cells, class-switched memory B cells; Tregs, regulatory T cells. *%* percentage, *#* c-counts, \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001.

**fIGURE 4** The counts of lymphocyte subpopulations and representative flow cytometry plots of adenoid tissues between AH-Molds+HDM+ (n=9) and AH-HDM+ (n=17) children. **a** B cells; **b** Naive B cells; **c** Memory B cells; **d** CSMB cells. **e-h** Representative flow cytometry plots of B cells, naive B cells, USMB cells, and CSMB cells in adenoid tissues between two groups. Memory B cells were composed of USMB and CSMB cells. Midlines indicate the median values. Statistical significances were determined using unpaired, two-tailed Mann-Whitney *U* test. AH-Molds+HDM+, children with AH polysensitized to molds and HDM; AH-HDM+, children with AH monosensitized to HDM; CSMB cells, class-switched memory B cells; USMB cells, un-switched memory B cells. % percentage, *#* c-counts, \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001.

**FIGURE S1** Subpopulation identification of natural killer cells, monocytes, and T cells according to the common extracellular markers. CD, Cluster of Differentiation; HLA-DR, human leukocyte antigen DR; NK cell, natural killer cell; NKT cell,natural killer T cell. + positive, - negative, high high expression, low low expression.

**FIGURE S2** Subpopulation identification of CD4+ and CD8+ T cells according to the common extracellular markers. CD, Cluster of Differentiation; CCR7, C-C chemokine receptor type 7; PD1, programmed cell death protein 1. + positive, - negative, high high expression, low low expression

**FIGURE S3** Subpopulation identification of B cells according to the common extracellular markers. CD, Cluster of Differentiation; IgD, Immunoglobulin D; IgM, Immunoglobulin M; CSMB cell, class-switched memory B cell; USMB cell, un-switched memory B cell. + positive, - negative, high high expression, low low expression.

**FIGURE S4** Subpopulation identification of T helper cells and regulatory T cells according to the common extracellular markers. CD, Cluster of Differentiation; Th, T helper cells; Treg, regulatory T cell. + positive, - negative, high high expression, low low expression.

**FIGURE S5** Representative flow cytometry plots of adenoid tissues inAH-AR and AH-nAR children. **a** NKTs; **b** CD56high NKs; **c** CD16+CD56+ NKs; **d** HLA-DR+ NKs; **e** B cells; **f** Naive B cells; **g** USMB cells; **h** CSMB cells; **i** Tregs; **j** Activated Tregs. Memory B cells were composed of USMB and CSMB cells. NKTs, natural killer T cells; NKs, natural killer cells;USMB cells, un-switched memory B cells; CSMB cells, class-switched memory B cells; Tregs, regulatory T cells.