Distinct inflammatory features and nasal T_H2 cell profiles in allergic patients with chronic rhinosinusitis

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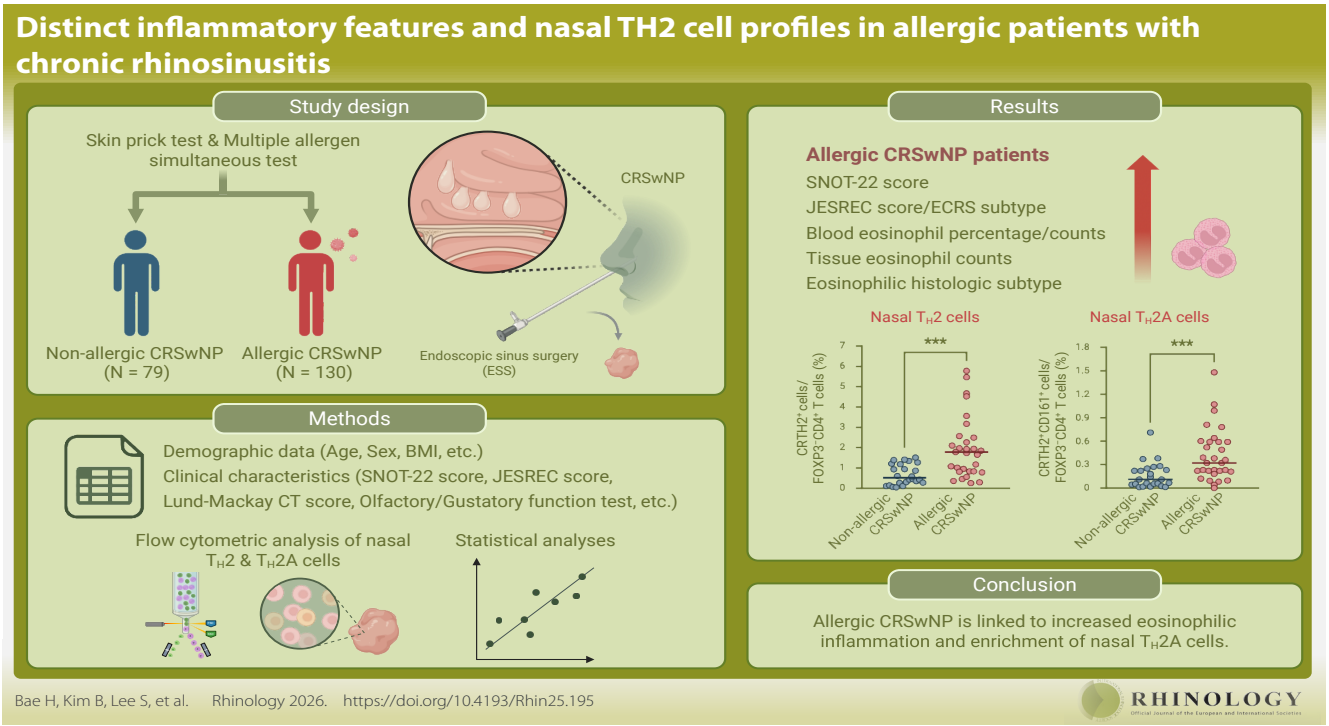

Abstract

Background: Various factors affect the immunopathogenesis of chronic rhinosinusitis (CRS). Although the relationship between allergies and CRS has attracted considerable attention, it remains controversial. Notably, little is known about whether the clinical characteristics and immunological profiles differ based on allergic sensitization status among patients with CRS.

Methodology: This study included 209 patients with CRS with nasal polyps (CRSwNP) who underwent endoscopic sinus surgery, and their nasal polyp tissues were obtained. Patients were divided into two groups according to allergic sensitization status: "allergic" and "non-allergic" groups. The clinical characteristics and immunological profiles were compared between the two groups. Ex vivo phenotypes of nasal $CD4^+$ T cells were analyzed using flow cytometry.

Results: Compared to the non-allergic group, the allergic group exhibited a significantly higher prevalence of comorbid asthma, serum total IgE levels, SNOT-22 scores, JESREC scores, blood/ tissue eosinophil counts, the proportion of eosinophilic histologic type, and lower gustatory function test scores. Additionally, the frequencies of nasal T helper 2 (T_H2) and proallergic T_H2 (T_H2A) cells were significantly higher in the allergic group than in both the non-allergic and the control groups, and these frequencies were significantly correlated with eosinophilic inflammation.

Conclusions: Our study demonstrates that allergic sensitization is closely associated with eosinophilic inflammation, as indicated by elevated levels of blood/tissue eosinophils and nasal T_H2A cells, and by worse symptom scores in CRSwNP. Given the distinct immunological features of allergic patients, considering allergic sensitization within nasal tissue when managing CRSwNP is crucial.

Key words: allergy, allergic sensitization, chronic rhinosinusitis, eosinophil, T helper 2 cell, proallergic T cell

Introduction

Chronic rhinosinusitis (CRS) is an inflammatory disease of the nasal cavity and paranasal sinuses that lasts for at least 12 weeks and causes symptoms, such as facial pain or pressure, nasal discharge, congestion, and hyposmia/anosmia [1]. Patterns of inflammation in CRS are highly heterogeneous [2]. Clinical features, including symptoms and treatment outcomes, vary across inflammatory endotypes [3, 4]. Additionally, multiple factors, such as allergen sensitization, systemic diseases or comorbidities, alterations in microbiome composition, and congenital factors, may affect the immunopathogenesis of CRS, leading to distinct inflammatory patterns [5].

Given the broad effects of allergies on patient health, the role of allergic sensitization in CRS and the association between CRS and other allergic diseases such as asthma and allergic rhinitis (AR) are important topics of research interest. A population-based study demonstrated that patients with CRS had a higher prevalence of asthma and AR before diagnosis than control subjects without CRS [6]. A significant association has been observed between atopy and CRS with nasal polyps (NPs). Patients with atopy are likely to have CRS with NPs (CRSwNP) [7], and the degree of allergic reactivity is associated with the radiologic severity on CT scan [8]. Bachert et al. reported increased levels of total and specific IgE levels in NPs [9]. In contrast, disease severity was not associated with atopic status in patients with CRS [10, 11]. Furthermore, the rate of positive skin prick test results did not statistically differ between patients with CRS and healthy controls [12]. Collectively, the relationship between allergen sensitization and CRS remains controversial [13]. Whether clinical characteristics and immunological profiles differ based on the allergic sensitization status among patients with CRSwNP remains unclear. In particular, little is known about the impacts of allergic sensitization on local inflammation and nasal $CD4^+$ T cell profiles in the nasal tissue of patients with CRSwNP.

In the present study, we examined the clinical and histologic characteristics of patients with CRSwNP based on their allergic sensitization status to identify the effects of allergic sensitization on CRSwNP. We conducted flow cytometry analysis of NP tissue and analyzed whether the nasal $CD4^+$ T cell populations differed according to allergic sensitization status among patients with CRSwNP. We also investigated the association between the frequency of proallergic $CD4^+$ T cells and various clinical features.

Materials and methods

Study subjects

A total of 209 patients with CRSwNP who underwent endoscopic sinus surgery (ESS) at Severance Hospital, Seoul, Republic of Korea between November 2021 and February 2024 were enrolled in this study. The inclusion criteria were as follows: 1) patients diagnosed with CRSwNP according to the European Position Paper on Rhinosinusitis and Nasal Polyps 2020 guidelines [14], and 2) patients who had undergone ESS. The exclusion criteria were as follows: 1) patients with antrochoanal polyps, fungal sinusitis, cystic fibrosis, or other autoimmune diseases; 2) patients who had received systemic corticosteroids, intranasal corticosteroids, or antibiotics within 1 month prior to tissue collection; and 3) patients who had been treated with biologics and immunosuppressants. NP tissue was obtained from patients during ESS. Additionally, control individuals without CRSwNP (n = 46) were included in the study. This study was reviewed and approved by the institutional review board of Severance Hospital (no. 4-2021-0573). Informed consent was obtained from all donors or legally authorized representatives of the participants under 18 years of age. This study was conducted in accordance with the principles of the Declaration of Helsinki.

Assessment of allergic sensitization

Allergic sensitization was evaluated using the skin prick test (SPT) or the multiple allergen simultaneous test (MAST). Skin prick test was performed with commercial extracts of the main airborne allergens, including house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), pollen (birch, oak, grass, ragweed, mugwort, elm, and English plantain), *Aspergillus fumigatus*, cat and dog dander, and German cockroaches. The MAST was performed using the AdvanSure AlloScreen (LG Life Sciences, Korea). In MAST, 61 different inhaled allergens were used, including tree pollen (tree mix, birch, alder, oak, Japanese cedar, etc.), grass pollen (grass mix, cultivated rye, Timothy grass, orchard, Bermuda grass, etc.), weed pollen (ragweed, mugwort, Japanese hop, etc.), fungi (*Alternaria, Aspergillus, Cladosporium, Penicillium*, etc.), house dust mites (*Mite-pteronyssinus*, *Mite-farinae*, house dust, etc.), animal epithelia (cat, dog, hamster, etc.), cockroach mix, and foods (milk, apple, peach, crab, etc.). The total serum IgE levels were also measured. A patient with a positive SPT or MAST for at least one allergen was defined as having allergic sensitization.

Demographic and clinical variables

Demographic and clinical characteristics, including sex, age, body mass index (BMI), asthma comorbidity, history of revision surgery, and CRS refractoriness, were obtained. According to the EPOS2020 guidelines [14], revision surgery was defined based on whether the patient had undergone endoscopic sinus surgery before sample collection. In addition, patients who did not reach an acceptable level of disease control (controlled or partially controlled) despite adequate surgery, intranasal corticosteroid treatment, and up to two short courses of antibiotics or systemic corticosteroids in the last year were considered to have refractory disease in line with the EPOS2020 guideline [14]. Blood laboratory tests were conducted in all included patients and control individuals. Information on the counts and percentages (%) of eosinophils, neutrophils, lymphocytes, monocytes, and

Table 1. Comparison of demographic and clinical characteristics between non-allergic and allergic CRSwNP patients.

Parameter	Non-allergic (n = 79)	Allergic (n = 130)	P value
Sex			0.028[c]
Male	51 (64.6%)	102 (78.5%)	
Female	28 (35.4%)	28 (21.5%)	
Age, years	50.43 (± 14.42)	46.26 (± 15.41)	0.053[a]
Body mass index (kg/m2)	24.75 (± 4.12)	25.08 (± 3.48)	0.315[b]
Asthma comorbidity, n (%)	9 (11.4%)	31 (23.8%)	0.026[c]
Revision, n (%)	21 (26.9%)	45 (34.6%)	0.249[c]
Refractoriness, n (%)	24 (30.4%)	48 (36.9%)	0.334[c]
SNOT-22 score	31.91 (± 18.59)	39.01 (± 21.75)	0.030[b]
Lund-Mackay CT score	14.05 (± 6.39)	15.16 (± 6.2)	0.307[b]
Olfactory function test score	16.74 (± 7.28)	16.48 (± 6.98)	0.887[b]
Gustatory function test score	17.81 (± 4.11)	16.23 (± 4.3)	0.010[b]
JESREC score	9.9 (± 4.62)	12.27 (± 4.21)	< 0.001[b]
JESREC subtype			
ECRS, n (%)	41 (51.9%)	102 (78.5%)	< 0.001[c]
Non-ECRS, n (%)	38 (48.1%)	28 (21.5%)	

CRSwNP, CRS with nasal polyp; Refractoriness, difficult to treat CRS; CT, computed tomography; ECRS, eosinophilic CRS; JESREC, Japanese epidemiological survey of refractory eosinophilic chronic rhinosinusitis study; SNOT-22, 22-item sino-nasal outcome test. Data are presented as number (%) or mean (± standard deviation). [a] Statistical analysis was performed using the Independent-T test. [b] Statistical analysis was performed using the Mann–Whitney U test. [c] Statistical analysis was performed using the Chi-squared test.

basophils was extracted.

The 22-item Sino-nasal Outcome Test (SNOT-22) score [15], Lund–Mackay CT score [16], and Japanese Epidemiological Survey of Refractory Eosinophilic Chronic Rhinosinusitis (JESREC) clinical scoring system [17, 18] were evaluated. The cut-off value for the JESREC score for ECRS was 11. If the JESREC score was 11 or higher, the patient was diagnosed with eosinophilic CRS (ECRS), while those with lower score were categorized as having non-eosinophilic CRS (NECRS) [17]. Olfactory function was evaluated using the YSK olfactory function test (YOF test; Kimex Co., Suwon, Republic of Korea), as described previously [19]. This test encompasses measures of odor threshold (T), discrimination (D), and identification (I), each with a maximum score of 12. The total TDI score was calculated by adding the T, D, and I scores, with a maximum score of 36. Additionally, the YSK gustatory function test (RHICO Medical Co., Seoul, Republic of Korea) was performed to assess five basic tastes: salty, sour, sweet, bitter, and umami [19].

For further details regarding the materials and methods used, please refer to the Supplementary Materials.

Results

Comparison of clinical characteristics between allergic and non-allergic CRS groups

In total, 209 patients with CRSwNP were included in this study. The mean (SD) age was 47.84 (15.14) years. The patients were categorized into two groups according to their allergic sensitization status: "allergic" (n = 130) and "non-allergic" groups (n = 79). We compared the demographic and clinical characteristics of the non-allergic and allergic groups (Table 1). Compared with the non-allergic group, the allergic group had a higher proportion of males (64.6% vs 78.5%, P = 0.028) and exhibited a significantly higher prevalence of comorbid asthma (11.4% vs 23.8%, P = 0.026). Additionally, the allergic group had significantly higher JESREC scores (9.9 vs 12.27, P < 0.001) and a higher frequency of ECRS group (51.9% vs 78.5%, P < 0.001) when patients were divided into two groups based on JESREC scores. Compared to the non-allergic group, the allergic group had a significantly higher SNOT-22 score (31.91 vs. 39.01, P = 0.030) and a lower gustatory function test score (17.81 vs. 16.23, P = 0.010). The proportion of revision surgery (26.9% vs 34.6%, P = 0.249), refractory disease (30.4% vs 36.9%, P = 0.334), and the Lund-Mackay CT score (14.05 vs 15.16, P = 0.307) did not differ between the non-allergic and allergic groups. No significant differences were also observed in age, BMI, and olfactory function test scores between the two groups.

Blood laboratory tests and tissue inflammatory cell infiltration in allergic and non-allergic CRS groups

Next, we compared the blood test results, including serum total

Table 2. Comparison of serum total IgE and blood parameters between allergic and non-allergic CRS patients.

Parameter	Non-allergic (n = 79)	Allergic (n = 130)	P value [a]
Serum total IgE (IU/mL)	68.36 (± 104.24)	346.59 (± 527.56)	< 0.001
Blood parameters			
Eosinophil, %	3.82 (± 3.30)	5.45 (± 3.98)	< 0.001
Eosinophil count, /μL	251.65 (± 231.76)	364.31 (± 279.8)	< 0.001
Neutrophil, %	57.9 (± 9.17)	54.38 (± 9.13)	0.008
Neutrophil count, /μL	4020.18 (± 1775.11)	3851.85 (± 1468.87)	0.495
Lymphocyte, %	31.22 (± 7.94)	32.27 (± 8.24)	0.363
Lymphocyte count, /μL	2065.7 (± 570.15)	2192.54 (± 670.92)	0.213
Monocyte, %	5.74 (± 1.47)	6.32 (± 1.87)	0.022
Monocyte count, /μL	387.47 (± 151.89)	429.38 (± 134.12)	0.019
Basophil, %	0.55 (± 0.31)	0.65 (± 0.33)	0.031
Basophil count, /μL	37.34 (± 22.4)	43.46 (± 22.81)	0.035

Data are presented as mean (± standard deviation). [a] Statistical analysis was performed using the Mann–Whitney U test.

Table 3. Comparison of histopathologic features between non-allergic and allergic CRSwNP patients.

Parameter	Non-allergic (n = 77)	Allergic (n = 129)	P value
Tissue eosinophil count (cells/HPF)	72.41 (± 115.61)	114.21 (± 149.3)	0.009 [b]
Tissue neutrophil count (cells/HPF)	15.89 (± 20.29)	31.02 (± 52.1)	0.296 [b]
Histologic subtype			
Eosinophilic, n (%)	34 (44.2%)	77 (59.7%)	0.03 [c]
Non-eosinophilic, n (%)	43 (55.8%)	52 (40.3%)	

HPF, high-power field. Data are presented as number (%) or mean (± standard deviation). [b] Statistical analysis was performed using the Mann–Whitney U test. [c] Statistical analysis was performed using the Chi-squared test.

IgE levels and the percentage/counts of eosinophils, neutrophils, lymphocytes, monocytes, and basophils (Table 2). Serum total IgE (68.36 vs 346.59 IU/mL, P < 0.001), eosinophil percentage (3.82% vs 5.45%, P < 0.001), eosinophil count (251.65 vs 364.31 cells/μL, P < 0.001), basophil percentage (0.55% vs. 0.65%, P = 0.031), and basophil count (37.34 vs. 43.46 cells/μL, P = 0.035) were significantly higher in the allergic group than in the non-allergic group. Furthermore, neutrophil percentage (57.9% vs. 54.38%, P = 0.008) was significantly lower in the allergic group than in the non-allergic group. When we compared the non-allergic group and control individuals without CRSwNP (control group), eosinophil percentage/count and neutrophil percentage/count were significantly higher in the non-allergic CRSwNP group than in the control group (Table S1).

Analysis of the tissue specimens (Table 3) revealed that the allergic group exhibited a higher tissue eosinophil count than the non-allergic group (72.41 vs 114.21 cells/HPF, P = 0.009). In line with this, the frequency of the eosinophilic histologic subtype was significantly higher in the allergic group than in the non-allergic group (44.2% vs 59.7%, P = 0.03). In contrast, no significant difference was observed in tissue neutrophil counts between the two groups.

We further classified the allergic CRSwNP patients into three subgroups based on allergen sensitization profiles: HDM-allergic, pollen-allergic, and double-allergic (sensitized to both HDM and pollen) and conducted comparative analyses (Tables S2 and S3). Serum total IgE levels increased in the following order: non-allergic < HDM-allergic < pollen-allergic < double-allergic (68.36 vs 211.88 vs 345.36 vs 593.12 IU/mL, P < 0.001). In addition, blood eosinophil percentages were significantly higher in all three allergic subgroups than in the non-allergic group (Table S2). Tissue eosinophil counts were significantly higher in the HDM-allergic subgroup than in the non-allergic group and tended to be higher in the pollen-allergic and double-allergic subgroups as well (Table S3). These findings indicate that all allergic subgroups exhibit more pronounced eosinophilic inflammation than the non-allergic subgroup, irrespective of allergen types.

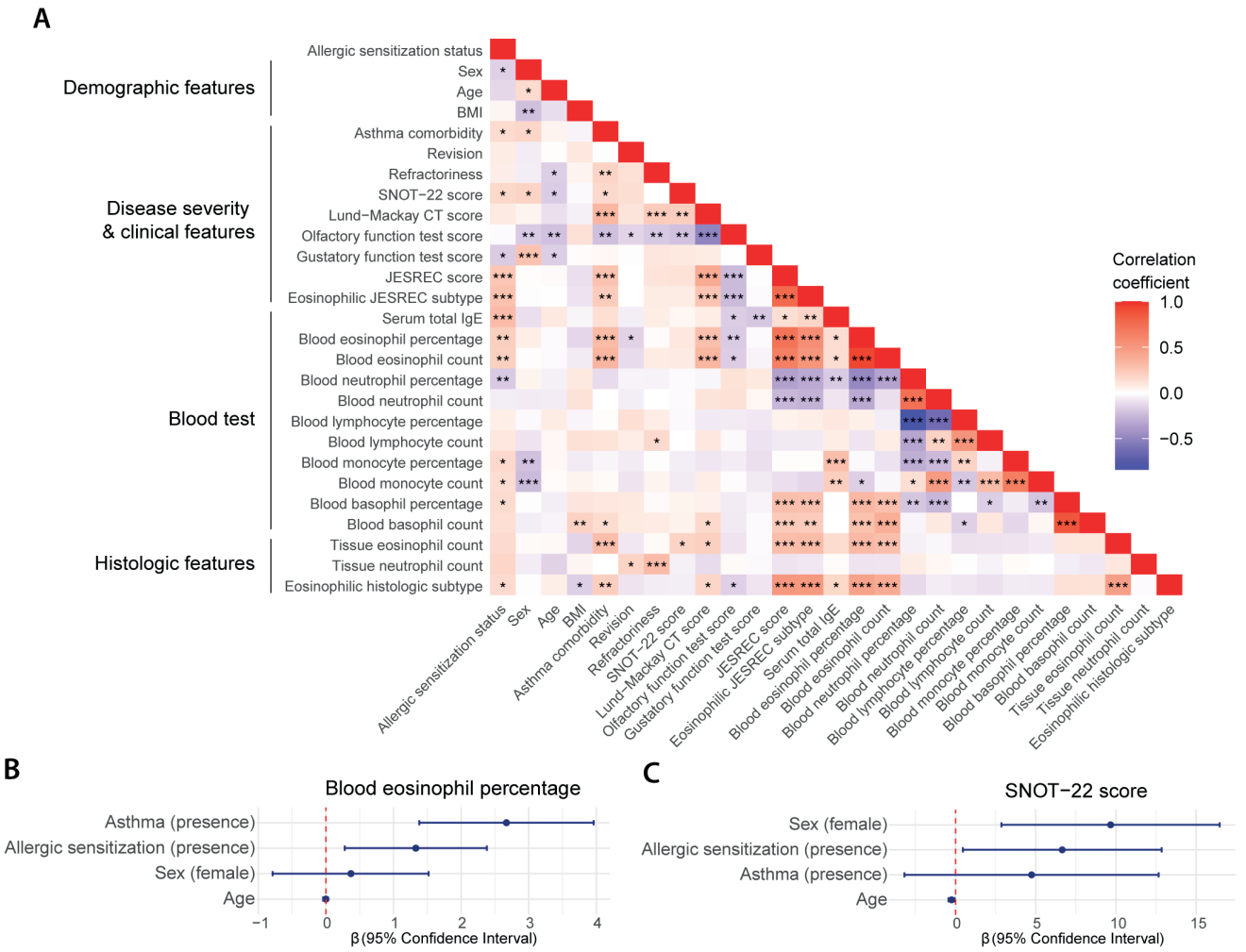

Figure 1. Association between allergic sensitization and other disease-related variables. (A) A heatmap depicting the correlation between allergic sensitization status and various disease-related variables in patients with CRSwNP (n = 209). The colors represent the magnitude of the correlation coefficients. *P < 0.05; **P < 0.01; ***P < 0.001. (B) Forest plots of the linear regression analysis showing regression coefficients (β) and 95% confidence intervals (CIs) for blood eosinophil percentage. (C) Forest plots of the linear regression analysis showing regression coefficients (β) and 95% CIs for the SNOT-22 score.

Factors associated with allergic sensitization in patients with CRS

To elucidate the factors associated with allergic sensitization in patients with CRS, we conducted a correlation analysis of the allergic sensitization status and serum total IgE levels with other variables (Figure 1A). The allergic sensitization status was significantly correlated with serum total IgE levels (P < 0.001), eosinophil percentages (P = 0.003), eosinophil counts (P = 0.003), monocyte percentages (P = 0.019), monocyte counts (P = 0.039), basophil percentages (P = 0.034), and JESREC scores (P < 0.001). In addition, the presence of allergic sensitization significantly increased the likelihood of having the eosinophilic JESREC subtype (P < 0.001), eosinophilic histologic subtype (P = 0.031), and asthma comorbidity (P = 0.026), as well as lower blood neutrophil percentages (P = 0.008). Furthermore, allergic sensitization was associated with more severe clinical symptoms, including higher SNOT-22 scores (P = 0.025) and worse gustatory function (P = 0.01). It was also more common in male sex (P = 0.028). Serum total IgE levels significantly positively correlated with various factors, not only allergic sensitization status but also the percentage (P = 0.039) and the count (P = 0.042) of blood eosinophils, the percentage (P < 0.001) and the count (P = 0.002) of monocytes, JESREC score (P = 0.049), eosinophilic JESREC subtype (P = 0.010), and eosinophilic histologic subtype (P = 0.016). In contrast, serum total IgE levels were inversely correlated with olfactory function test score (P = 0.015), gustatory function test score (P = 0.005), and percentage of neutrophils (P = 0.008).

Association of allergen sentization with eosinophilic inflammation and symptom severity

The clinical association between asthma and CRSwNP is well established [6, 20] and asthma is known to be closely associated with

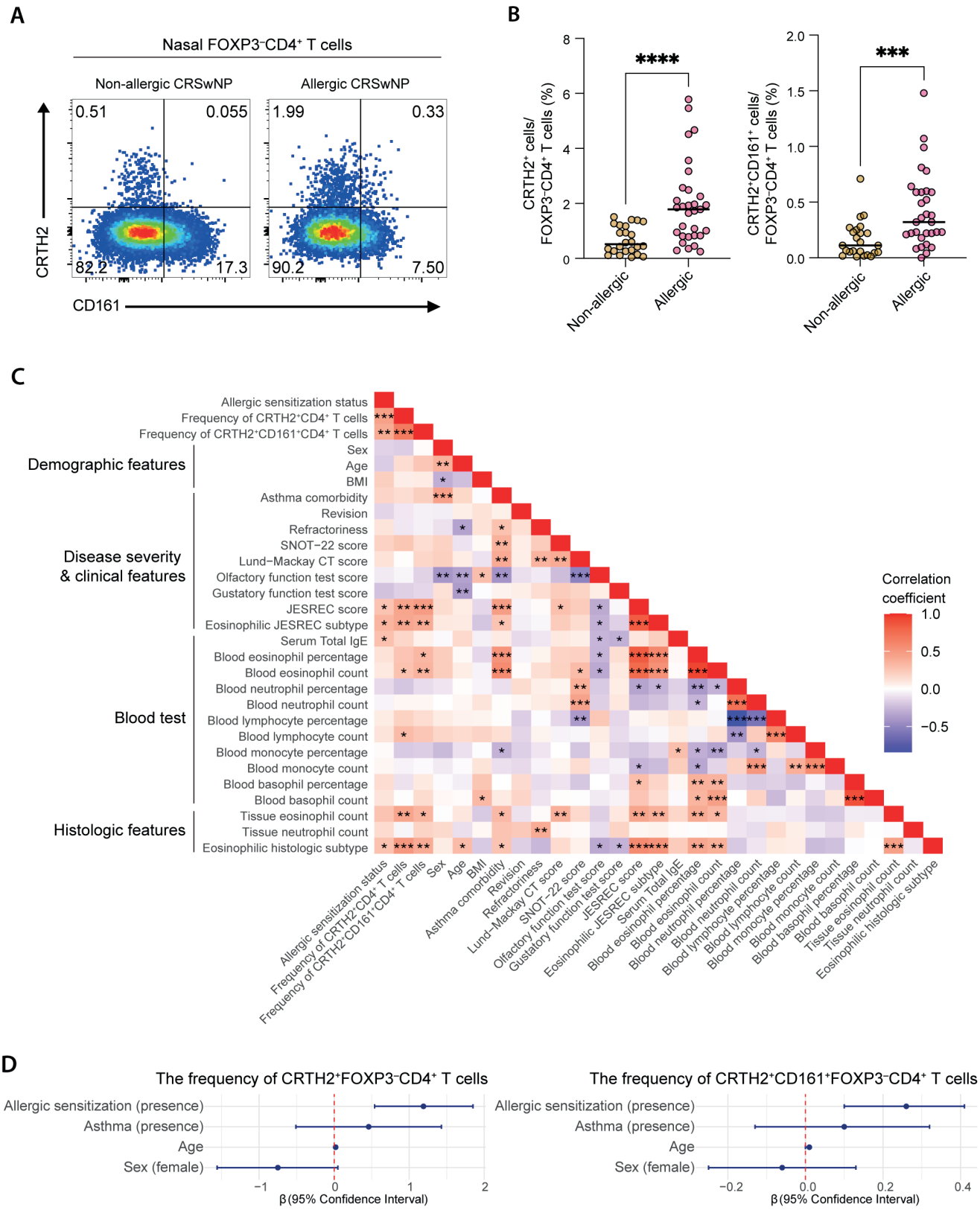

Figure 2. Analyses of nasal CD4⁺ T cells in allergic and non-allergic patients with CRSwNP. (A and B) Representative flow cytometry plots (A) and summary (B) of the frequencies of T_H2 (CRTH2⁺) and T_H2A (CRTH2⁺CD161⁺) cells among nasal FOXP3⁻CD4⁺ T cells in non-allergic (n = 24) and allergic (n = 31) CRSwNP groups. (C) A heatmap illustrating the correlation coefficients between the frequencies of T_H2 and T_H2A cells and other variables in patients with CRSwNP (n = 55). The colors represent the magnitude of the correlation coefficients. *P < 0.05; **P < 0.01; ***P < 0.001. (D) Forest plots of the linear regression analyses showing regression coefficients (β) and 95% confidence intervals (CIs) for the frequencies of T_H2 and T_H2A cells.

allergy and type 2 inflammation. Therefore, its presence may significantly confound the observed correlations. However, even after excluding patients with asthma from the analysis, allergic patients with CRSwNP still exhibited significantly greater eosinophilic inflammation, characterized by higher blood eosinophil percentage and count, elevated tissue eosinophil counts, and a higher frequency of the eosinophilic histologic subtype, than non-allergic patients with CRSwNP (Tables S4 and S5).

To further determine whether allergic sensitization is independently associated with eosinophilic inflammation, we conducted regression analyses adjusted for asthma comorbidity, sex, and age. Allergic sensitization was significantly associated with higher blood eosinophil percentage [β (regression coefficient) = 1.33, 95% confidence interval (CI) = 0.28 – 2.38] (Figure 1B). Since the allergic group exhibited higher SNOT-22 scores (Table 1), we additionally conducted a linear regression analysis to further confirm the impact of allergic sensitization on clinical symptoms. We found that allergic sensitization was significantly associated with higher SNOT-22 scores (β = 6.65, 95% CI = 0.45 – 12.8) after adjusting for asthma comorbidity, sex, and age (Figure 1C). Additionally, female sex was independently associated with higher SNOT-22 scores compared to male sex (β for female to male = 9.67, 95% CI = 2.86 – 16.50), while increasing age was correlated with a slight but significant decrease in symptom severity (β = -0.25, 95% CI = -0.44 – -0.06). These findings suggest that allergic sensitization is an independent factor influencing symptom severity in patients with CRSwNP, even after accounting for asthma comorbidity and demographic variables.

Analysis of ex vivo phenotypes of nasal CD4$^+$ T cells in allergic and non-allergic CRS groups

We compared ex vivo phenotypes of the nasal CD4$^+$ T cells between the two groups using flow cytometry (Figure S1). In humans, T helper 2 (T_H2) cells can be identified by the expression of the prostaglandin D2 receptor CRTH2 (also known as DP2 or CD294). Additionally, proallergic T_H2 cells, denoted as T_H2A cells, are characterized by the coexpression of CRTH2 and CD161 (C-type lectin-like type-II transmembrane protein) [21, 22]. We analyzed the frequencies of T_H2 (CRTH2$^+$ cells) and T_H2A cells (CRTH2$^+$CD161$^+$ cells) among non-regulatory FOXP3$^-$CD4$^+$ T cells in nasal tissues from non-allergic and allergic CRSwNP patients (Figure 2A). The frequencies of CRTH2$^+$ cells and CRTH2$^+$CD161$^+$ cells among nasal FOXP3$^-$CD4$^+$ T cells were significantly higher in the allergic group than in the non-allergic CRSwNP group (Figure 2B), and this difference remained significant even after excluding patients with asthma (Figure S2).

Next, we examined the correlation between the frequencies of these nasal CD4$^+$ T-cell subsets and various variables in patients with CRSwNP (Figure 2C). Allergic sensitization was positively correlated with the frequencies of CRTH2$^+$ cells (P < 0.001) and CRTH2$^+$CD161$^+$ cells (P = 0.001) among nasal FOXP3$^-$CD4$^+$ T cells. Additionally, the frequencies of CRTH2$^+$ and CRTH2$^+$CD161$^+$ cells among nasal FOXP3$^-$CD4$^+$ T cells were positively correlated with the JESREC score (P = 0.001 , P < 0.001), eosinophilic JESREC subtype (P = 0.002, P = 0.004), blood eosinophil count (P = 0.042, P = 0.009), tissue eosinophil count (P = 0.002 , P = 0.016), and eosinophilic histologic subtype (P < 0.001, P = 0.004). To investigate whether allergic sensitization is independently associated with nasal T_H2 and T_H2A cells of CRSwNP patients, we further conducted linear regression analyses adjusting for sex, age, and asthma comorbidity. Allergic sensitization was significantly associated with the frequencies of CRTH2$^+$ (β = 1.19, 95% CI = 0.54 – 1.85) and CRTH2$^+$CD161$^+$ (β = 0.26, 95% CI = 0.10 – 0.41) cells among nasal FOXP3$^-$CD4$^+$ T cells, independent of asthma comorbidity (Figure 2D). Collectively, these results suggest that T_H2 and T_H2A cells, which are significantly associated with eosinophilic inflammation, are enriched in the NP tissue of CRSwNP patients with allergic sensitization.

Discussion

CRS is a heterogeneous disease with a wide range of clinical manifestations and immunological features. Diverse factors contribute to the development and progression of CRS. Allergies are potential contributors to CRS; however, their relationship and its underlying mechanisms remain unclear [13]. In this study, we aimed to elucidate the relationship between allergies and CRSwNP by examining the clinical and histologic characteristics of CRS based on allergic sensitization. We demonstrated that patients with allergic sensitization showed pronounced eosinophilic inflammation and possessed higher frequencies of proallergic T_H2A cells in the nasal tissue than those without allergic sensitization. Considering that patients with allergic sensitization exhibit distinct features, clinicians should consider the presence of allergic sensitization when managing patients with CRSwNP.

In the present study, we observed that allergic sensitization was strongly associated not only with total serum IgE, but also with eosinophilic inflammation-related features, including asthma comorbidity, higher JESREC score, increased ratio of ECRS JESREC subtype, elevated blood eosinophil counts and percentages, and higher tissue eosinophil counts. Given that biologics targeting type 2 inflammation such as dupilumab, omalizumab, and mepolizumab are currently available for the management of CRS, our findings suggest that these biologics may be more effective in patients with allergic sensitization.

We also compared ex vivo phenotypes of nasal CD4$^+$ T cells in allergic and non-allergic groups. Our results revealed that CRTH2$^+$CD4$^+$ and CRTH2$^+$CD161$^+$CD4$^+$ T cells were more abundant in nasal tissues of the allergic group than in those of the non-allergic group. These findings indicate that localized enrichment of T_H2 and T_H2A cells is associated with allergic sensitization in patients with CRSwNP. To the best of our knowledge,

this is the first study to analyze the frequencies of T_H2 and T_H2A cells in the nasal tissue of patients with CRSwNP according to their allergic sensitization status. A recent study using mass cytometric analysis of peripheral blood mononuclear cells showed that the frequency of circulating T_H2A cells was significantly higher in allergic individuals than in non-allergic individuals [23]. However, they found no significant differences in the frequency of circulating T_H2A cells between allergic and non-allergic patients with CRS. The discrepancy with our results may be due to differences in sample types. As CRS is primarily considered a localized disease, the tissue inflammatory microenvironment may recruit T_H2 and T_H2A cells, which are increased in allergic patients, to nasal tissue. Further research should focus on tissue-specific allergic inflammation to better understand the association between allergies and CRS. It would be interesting to analyze other immune cells associated with allergic inflammation, such as IgE-producing plasma cells, and their interaction with T_H2A cells within the nasal tissue of CRS patients with allergic sensitization.

In addition, we investigated whether the frequency ofT_H2A cells was correlated with other clinico-histological features in patients with CRSwNP. Remarkably, the frequencies of nasal T_H2 and T_H2A cells were positively correlated with higher JESREC scores, higher blood eosinophil counts, and eosinophilic histologic subtypes, suggesting their involvement in eosinophilic inflammation. Considering that humanT_H2A cells play a critical role in T_H2-driven pathology by simultaneously producing multiple type 2 cytokines, including IL-4, IL-5, and IL-13 [21], these findings indicate that nasal $CD4^+$ T cells are skewed toward proallergic $CD4^+$ T cells (T_H2A cells) in allergic individuals, which may contribute to prominent eosinophilic/type 2 inflammation. Therefore, currently available biologics targeting type 2 cytokines may be particularly beneficial for allergic CRSwNP patients by modulating the effector functions of T_H2A cells. In this context, the presence of allergic sensitization may be an important consideration for implementing precision medicine and tailoring biologic therapies in CRSwNP. However, further prospective studies are required to evaluate whether the efficacy of biologics differs between allergic and non-allergic patients with CRSwNP. We observed no significant association between the frequency of T_H2A cells and disease refractoriness, suggesting that refractoriness may not be solely driven by the abundance of T_H2A cells. The precise pathogenic role of T_H2A cells in allergic CRSwNP, along with their underlying mechanisms, should be investigated in future studies.

The relationship between allergies and disease or symptom severity and progression in CRS has been controversial [5, 10, 11, 13]. In this study, we found that the allergic group had significantly higher SNOT-22 scores compared to the non-allergic group. The linear regression analyses further demonstrated a significant impact of allergic sensitization on symptom severity. These findings suggest that CRSwNP patients with allergic sensitization experience a greater symptom burden and more pronounced impairment in quality of life. Further studies are required to validate these results and elucidate the mechanisms underlying these clinical features, with a particular focus on the impact of localized eosinophilic inflammation. However, the proportion of revision history, refractory disease, and Lund-Mackay CT scores did not differ between the allergic and non-allergic groups, indicating that disease progression or recurrence is not solely influenced by allergic sensitization. These findings are consistent with and prior studies, suggesting that although allergic sensitization is significantly associated with eosinophilic infiltration, it has a limited effect on clinical symptoms and underlying mechanisms [11, 24].

Our study has several limitations. First, allergic sensitization status was determined based solely on sensitization test results, and the clinical relevance of sensitization could not be fully assessed, as most patients presented with CRS-related symptoms independent of allergen exposure. Second, the cross-sectional design and single time-point sampling of nasal tissue and peripheral blood precluded the evaluation of seasonal variation in immune cell or marker profiles in relation to allergen season.

Conclusion

Our study revealed a relationship between allergic sensitization and CRS, particularly in the context of local immunological features, including tissue eosinophil infiltration and nasal T_H2A cells. The current investigation demonstrated that allergic sensitization is strongly associated with worse symptom scores, increased eosinophilic inflammation, and enrichment of proallergic T_H2A cells in the nasal tissue. These findings significantly advance our understanding of the effect of allergic sensitization on CRS pathogenesis and provide valuable insights into the management of allergic CRSwNP.

Conflict of interest

The authors declare no conflict of interest.

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Authors contributions

HB designed the study, analyzed the data, and wrote the manuscript; GK, SL, and M-SK performed the experiments and analyzed the data; H-JC and C-HK provided clinical samples and analyzed the data; M-SR designed the study, provided clinical samples, analyzed the data, wrote the manuscript, and reviewed the manuscript.

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SUPPLEMENTARY MATERIAL

Supplementary Materials

Assessment of allergen types

For subanalyses based on allergen type, patients were classified as sensitized to HDM allergens if they showed a positive response to house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) on SPT, or to house dust mite-related allergens (e.g., *Mite-pteronyssinus*, *Mite-farinae*, house dust) on MAST. Sensitization to pollen allergens was defined as a positive response to pollen allergens (birch, oak, grass, ragweed, mugwort, elm, and English plantain) on SPT, or to tree pollens (e.g., tree mix, birch, alder, oak, and Japanese cedar), grass pollens (e.g., grass mix, cultivated rye, Timothy grass, orchard grass, and Bermuda grass), or weed pollens (e.g., ragweed, mugwort, and Japanese hop) on MAST.

Immunohistochemistry and histologic evaluation of nasal tissue

Immunostaining was performed as follows. After paraffin removal, the slides were boiled in an antigen retrieval solution (IHC World, #IW-1100) in a steaming bowl for 40 min. After cooling, the slides were incubated with 3% hydrogen peroxide (Dako (Agilent Technologies, Carpinteria, CA, USA) #S2023) for 10 min. After washing with phosphate buffered saline (PBS), the slides were blocked with 5% bis(trimethylsilyl)acetamide for 1 h and incubated with a primary antibody for 1 h at room temperature. Human neutrophil elastase/ELA2 antibody (R&D systems, Minneapolis, MN, USA, #MAB91671, 1:500) was used as the primary antibody. After washing with fetal bovine serum (FBS), the slides were incubated with an anti-horseradish peroxidase (#K4001; Dako) secondary antibody for 30 min at room temperature. After washing with PBS, the slides were incubated with 3,3'-diaminobenzidine (DAB; Dako, #K4003, 1:100) for 1 min to allow chromogenic development, rinsed with tap water, and counterstained with hematoxylin for 20 s to visualize the nuclei. After a final wash with tap water for 5 min and distilled water for 3 min, the slides were mounted using a synthetic mounting medium (Thermo Fisher Scientific, Waltham, MA, USA, #6769007) with DAB.

In tissue samples stained with hematoxylin and eosin, eosinophil counts were quantified in five high-power fields (HPFs) at ×400 magnification. Neutrophils were quantified as human neutrophil elastase-positive cells. According to previous studies [25-27], the histologic subtype was categorized into two groups: eosinophilic (eosinophils > 10% of the inflammatory cells) and non-eosinophilic (eosinophils ≤ 10% of the inflammatory cells).

Nasal cell isolation and multicolor flow cytometry

For nasal cell isolation, NP tissue was mechanically dissociated, and then single-cell suspensions were prepared using a Tumor Dissociation Kit (Miltenyi Biotec, Auburn, CA, USA) with a gentleMACS dissociator (Miltenyi Biotec) and a 70-μm cell strainer (SPL Life Sciences, Gyeonggido, Korea). After isolation, the cells were cryopreserved in FBS (Corning) supplemented with 10% dimethyl sulfoxide (Sigma-Aldrich, St Louis, MO, USA) until use. The nasal cells were stained with fluorochrome-conjugated antibodies against specific surface markers for 10 min at room temperature. Dead cells were excluded using the LIVE/DEAD near-IR fluorescent reactive dye (Invitrogen, Carlsbad, CA, USA). Multicolor flow cytometry was performed using a Fortessa or Lyric instrument (BD Biosciences, San Jose, CA, USA), and the data were analyzed using FlowJo software (FlowJo LLC, Ashland, OR, USA).

We used the following fluorochrome-conjugated monoclonal antibodies (all from BD Biosciences): anti-CD14 APC-Cy7 (1:100 dilution, clone MφP9, catalog no. 557831); anti-CD3 BV510 (1:100 dilution, clone UCHT1, catalog no. 563109); anti-CD4 Alexa Fluor 700 (1:100 dilution, clone RPA-T4, catalog no. 557922); anti-CRTH2 APC-Cy7 (1:100 dilution, clone SJ25C1, catalog no. 557791); anti-CD161 BV605 (1:100 dilution, clone SK1, catalog no. 564116).

Statistical analysis

Data are expressed as mean and ± standard deviation (SD). When comparing the two groups, the significance of continuous variables was assessed using the independent t-test or the Mann–Whitney U test. The Chi-square test was used to analyze categorical variables. When comparing more than three groups, the significance of continuous variables was assessed using the Kruskal–Wallis test, followed by Dunn's test with Bonferroni adjustment. The Spearman's rank correlation test was used to assess the relationships between parameters. The linear and logistic regression analyses were performed to evaluate the impact of allergic sensitization on eosinophilic inflammation or clinical outcomes, adjusting for asthma comorbidity and demographic variables such as sex and age. P-values of less than 0.05 were considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics (version 28.0.0.0), R software (version 4.4.0), and GraphPad Prism (version 9.5.1).

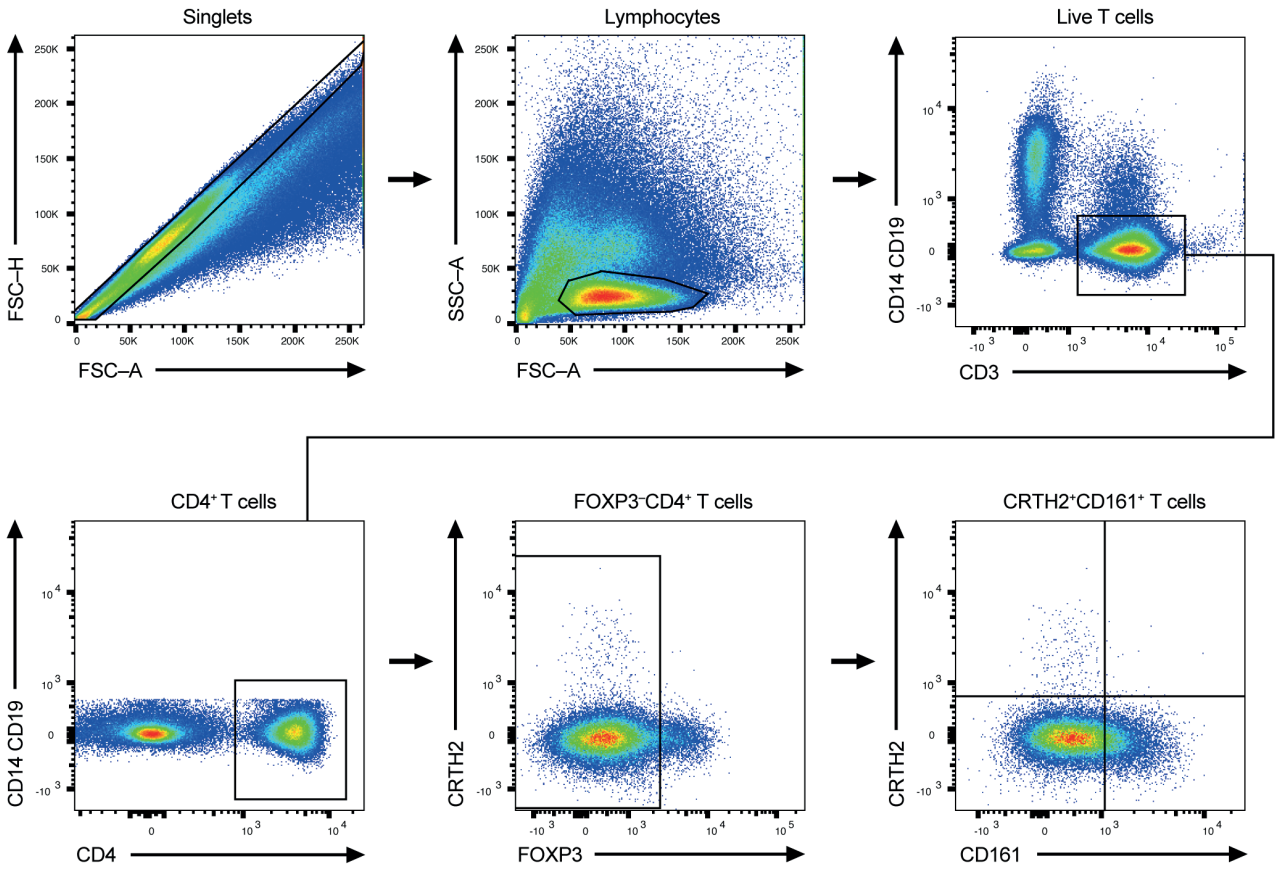

Figure S1. Gating strategy for the analysis of nasal CD4⁺ T cells. Representative flow cytometry gating strategy used for analyzing nasal T_H2 (CRTH2⁺) and T_H2A (CRTH2⁺CD161⁺) cells.

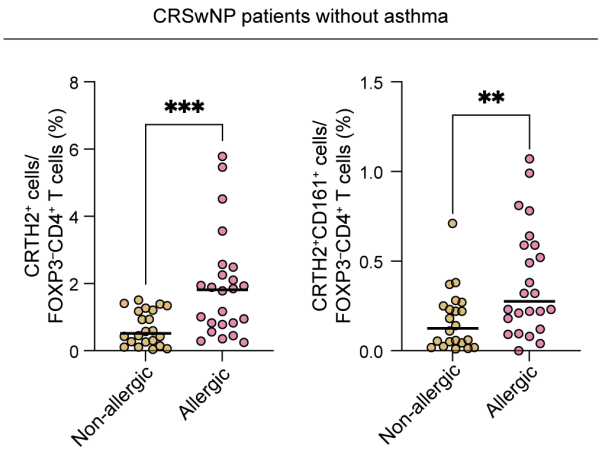

Figure S2. Analysis of nasal CD4⁺ T cells in allergic and non-allergic CRSwNP patients without asthma. Summary of the frequencies of T_H2 (CRTH2⁺) and T_H2A (CRTH2⁺CD161⁺) cells among nasal FOXP3⁻CD4⁺ T cells in allergic (n = 24) and non-allergic (n = 22) CRSwNP patients without asthma.

Table S1. Comparison of serum total IgE and blood parameters between control and non-allergic CRS patients.

Parameter	Control (n = 46)	Non-allergic (n = 79)	P value [a]
Serum total IgE (IU/mL)	46.09 (± 90.71)	68.36 (± 104.24)	0.033
Blood parameters			
Eosinophil, %	2.24 (± 1.26)	3.82 (± 3.30)	0.024
Eosinophil count, /μL	140.00 (± 91.83)	251.65 (± 231.76)	0.005
Neutrophil, %	53.54 (± 8.43)	57.90 (± 9.17)	0.025
Neutrophil count, /μL	3368.67 (± 1110.84)	4020.18 (± 1775.11)	0.018
Lymphocyte, %	36.48 (± 8.32)	31.22 (± 7.94)	0.003
Lymphocyte count, /μL	2223.11 (± 550.24)	2065.70 (± 570.15)	0.098
Monocyte, %	6.22 (± 1.70)	5.74 (± 1.47)	0.140
Monocyte count, /μL	385.78 (± 136.64)	387.47 (± 151.89)	0.935
Basophil, %	0.58 (± 0.18)	0.55 (± 0.31)	0.261
Basophil count, /μL	35.56 (± 11.59)	37.34 (± 22.40)	0.861

Data are presented as mean (± standard deviation). [a] Statistical analysis was performed using the Mann–Whitney U test.

Table S2. Comparison of serum total IgE and blood parameters among allergen sensitization subgroups.

Parameter	Non-allergic (n = 79)	HDM-allergic (n = 60)	Pollen-allergic (n = 12)	Double-allergic (n = 43)	P value [a]
Serum total IgE (IU/mL)	68.36 (± 104.24) [*,†,¶]	211.88 (± 291.12) [*,*]	345.36 (± 243.06) [†]	593.12 (± 776.62) [¶,*]	< 0.001
Blood parameters					
Eosinophil, %	3.82 (± 3.30) [*,†,¶]	5.42 (± 4.37) [*]	6.21 (± 3.59) [†]	5.52 (± 3.70) [¶]	0.001
Eosinophil count, /μL	251.65 (± 231.76) [*,†]	365.17 (± 309.45) [*]	384.17 (± 206.51) [†]	370.93 (± 275.95)	0.001
Neutrophil, %	57.90 (± 9.17)	54.46 (± 9.48)	52.50 (± 7.91)	54.20 (± 9.85)	0.091
Neutrophil count, /μL	4020.18 (± 1775.11)	3861.83 (± 1426.34)	3220.83 (± 798.82)	3836.05 (± 1620.79)	0.351
Lymphocyte, %	31.22 (± 7.94)	31.91 (± 9.04)	33.33 (± 8.82)	32.34 (± 7.56)	0.843
Lymphocyte count, /μL	2065.70 (± 570.15)	2173.83 (± 710.88)	2066.67 (± 533.55)	2162.56 (± 612.07)	0.807
Monocyte, %	5.74 (± 1.47)	6.15 (± 1.64)	6.83 (± 2.35)	6.48 (± 2.18)	0.108
Monocyte count, /μL	387.47 (± 151.89)	417.33 (± 112.84)	430.00 (± 162.48)	439.53 (± 162.50)	0.214
Basophil, %	0.55 (± 0.31)	0.69 (± 0.32)	0.63 (± 0.33)	0.62 (± 0.31)	0.069
Basophil count, /μL	37.34 (± 22.40)	45.50 (± 22.23)	40.83 (± 25.03)	41.16 (± 20.84)	0.057

Data are presented as mean (± standard deviation). [a] Statistical analysis was performed using the Kruskal–Wallis test. Dunn's multiple comparison test with Bonferroni adjustment was used for post-hoc analysis. Presentation of post-hoc test results : [*] $P < 0.05$ for the non-allergic vs. HDM-allergic group; [†] $P < 0.05$ for the nonallergic vs. pollen-allergic group; [¶] $P < 0.05$ for the non-allergic vs. double allergic group; * $P < 0.05$ for the HDM-allergic vs. double allergic group.

Table S3. Comparison of histopathologic features among allergen sensitization subgroups.

Parameter	Non-allergic (n = 77)	HDM-allergic (n = 60)	Pollen-allergic (n = 12)	Double-allergic (n = 42)	P value
Tissue eosinophil count (cells/HPF)	72.41 (± 115.61) *	119.03 (± 135.20) *	161.61 (± 217.74)	117.96 (± 161.22)	0.018 [a]
Tissue neutrophil count (cells/HPF)	15.89 (± 20.29)	35.08 (± 57.15)	22.57 (± 16.10)	33.07 (± 61.95)	0.259 [a]
Histologic subtype					
Eosinophilic, n (%)	34 (44.2%)	37 (61.7%)	8 (66.7%)	23 (54.8%)	0.166 [b]
Non-eosinophilic, n (%)	43 (55.8%)	23 (38.3%)	4 (33.3%)	19 (45.2%)	

Data are presented as mean (± standard deviation). [a] Statistical analysis was performed using the Kruskal–Wallis test. [b] Statistical analysis was performed using the Chi-square test. Dunn's multiple comparison test with Bonferroni adjustment was used for post-hoc analysis. Presentation of post-hoc test results : * $P < 0.05$ for the non-allergic vs. HDM-allergic group.

Table S4. Comparison of serum total IgE and blood parameters between non-allergic and allergic CRSwNP patients without asthma.

Parameter	Non-allergic (n = 70)	Allergic (n = 99)	P value
Serum total IgE (IU/mL)	61.49 (± 80.90)	317.77 (± 515.08)	< 0.001[b]
Blood parameters			
Eosinophil, %	3.39 (± 2.75)	4.88 (± 3.8)	0.001[b]
Eosinophil count, /μL	214.71 (± 169.28)	322.73 (± 265.65)	< 0.001[b]
Neutrophil, %	58 (± 9.47)	55 (± 9.18)	0.04[a]
Neutrophil count, /μL	4002.77 (± 1881.32)	3850.2 (± 1460.23)	0.705[b]
Lymphocyte, %	31.53 (± 8.26)	32.03 (± 8.59)	0.706[a]
Lymphocyte count, /μL	2056.14 (± 575.27)	2147.88 (± 664.15)	0.395[b]
Monocyte, %	5.83 (± 1.49)	6.33 (± 1.57)	0.04[b]
Monocyte count, /μL	389.86 (± 157.75)	427.58 (± 125.82)	0.04[b]
Basophil, %	0.53 (± 0.29)	0.65 (± 0.35)	0.021[b]
Basophil count, /μL	34.71 (± 19.17)	42.93 (± 23.74)	0.024[b]

Data are presented as mean (± standard deviation). [a] Statistical analysis was performed using the Independent T-test. [b] Statistical analysis was performed using the Mann–Whitney U test.

Table S5. Comparison of histopathologic features between non-allergic and allergic CRSwNP patients without asthma.

Parameter	Non-allergic (n = 69)	Allergic (n = 99)	P value
Tissue eosinophil count (cells/HPF)	48.24 (± 77.76)	98.29 (± 135.29)	0.002[a]
Tissue neutrophil count (cells/HPF)	16.9 (± 21.21)	31.28 (± 52.35)	0.454[a]
Histologic subtype			
Eosinophilic, n (%)	27 (36.6%)	55 (56.1%)	0.036[b]
Non-eosinophilic, n (%)	42 (63.4%)	44 (43.9%)	

HPF, high-power field. Data are presented as number (%) or mean (± standard deviation). [a] Statistical analysis was performed using the Mann–Whitney U test. [b] Statistical analysis was performed using the Chi-squared test.