Cross-talk between TH2 and TH17 pathways in patients with chronic rhinosinusitis with nasal polyps

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GRAPHICAL ABSTRACT

Background: Chronic rhinosinusitis with nasal polyps (CRSwNP) is a heterogeneous disease with a spectrum of endotypes. TH2- and TH17-related cytokines are 2 central regulators involved in the inflammation associated with CRSwNP.

Objective: We sought to investigate the interregulation of TH2 and TH17 pathways in Chinese patients with CRSwNP.

Methods: Levels of key TH2- and TH17-related factors were measured in homogenates of polyp tissue obtained from patients with CRSwNP. The relationship of these factors and their environmental stress/Pollution/Pathogen

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expression in groups classified according to tissue IL-5 and IL-17 concentrations were analyzed. Cross-regulation of TH2 and TH17 cytokines and the effects of dexamethasone treatment were studied in dispersed nasal polyp cells. Associations between TH2- and TH17 related factors and comorbid atopic status and asthma, disease recurrence, and edema scores were also explored.

Results: Four CRSwNP groups were classified based on expression or nonexpression of mutually exclusive TH2- and TH17-related factors. The TH2 cytokines IL-4 and IL-13 inhibited expression of TH17-related factors, whereas the TH17 cytokines IL-17 and TGF-β1 enhanced expression of TH2-related factors. Dexamethasone treatment inhibited both the TH2 and TH17 pathways. A patient’s atopic status was related to their TH2 immune response. Edema scores were positively correlated with the TH2 pathway and negatively correlated with the TH17 pathway.

Conclusion: The TH2 and TH17 pathways are mutually exclusive and regulate each other, favoring the development of a TH2 immune response in Chinese patients with CRSwNP. (J Allergy Clin Immunol 2019;144:1254-64.)

Key words: Chronic rhinosinusitis with nasal polyps, TH2, TH17, endotypes, shift

Abbreviations used

- CRS: Chronic rhinosinusitis
- CRSwNP: Chronic rhinosinusitis with nasal polyps
- DEX: Dexamethasone
- DNPC: Dispersed nasal polyp cell
- ECP: Eosinophil cationic protein
- G-CSF: Granulocyte colony-stimulating factor
- IP-10: Interferon-inducible protein 10
- MCA: Multiple correspondence analysis
- MPO: Myeloperoxidase
- NP: Nasal polyp
- TH: T helper
- IL: Interleukin
- G-CSF: Granulocyte colony-stimulating factor
- ECP: Eosinophil cationic protein
- MPO: Myeloperoxidase
- TH2: T helper 2
- TH17: T helper 17
- IL-5: Interleukin 5
- IL-17: Interleukin 17
- TGF-β: Transforming growth factor β
- DNPC: Dispersed nasal polyp cell
- IP-10: Interferon-inducible protein 10
- MPO: Myeloperoxidase
- NP: Nasal polyp
- TH2: T helper 2
- TH17: T helper 17
- IL-5: Interleukin 5
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- TGF-β: Transforming growth factor β
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- MPO: Myeloperoxidase
- NP: Nasal polyp
- TH2: T helper 2
- TH17: T helper 17
- IL-5: Interleukin 5
- IL-17: Interleukin 17
- TGF-β: Transforming growth factor β

Peripheral blood eosinophil and neutrophil analysis

Peripheral blood eosinophil and neutrophil numbers were measured with a standard automated cell counter.

Histologic analysis

Paraffin-embedded tissue sections were stained with hematoxylin and eosin and assessed for eosinophil and neutrophil counts and edema. Numbers of eosinophils and neutrophils were counted in 10 randomly selected high-power magnification fields (×400) and expressed as mean numbers of cells per high-power field. Edema was evaluated semiquantitatively according to the study of Zhang et al. by scoring on a scale of 0 to 2, where a rating of 0 indicated no edema, 1 indicated mild/moderate edema, and 2 indicated intense edema. Edema was scored in 5 randomly selected high-power magnification fields (×200) by an observer blinded to the study protocol and expressed as the mean score. Considering the subjective nature of this scoring system, analysis with ImageJ software (National Institutes of Health, Bethesda, Md) was further performed on 50% of randomly selected samples (48 from the 95 patients with CRSwNP) for objective evaluation of edema with ImageJ software. Because edema is defined as an abnormal accumulation of fluid (seen as white areas) in the extravascular and extraglandular compartments of NP tissue, ImageJ analysis was performed in the edematous areas in 10 high-power magnification fields (×200) in randomly selected pictures of hematoxylin and eosin–stained
samples. The edematous areas were graded with ImageJ software and expressed as mean percentages of the total area measured in each high-power field. The consistency of the subjective and objective measures of edema was further evaluated by analyzing the correlation between the 2 measures using Spearman correlation analysis.

**Picrosirius red staining for collagen**

Collagen content in NP tissue was assessed by using picrosirius red staining, as previously described. For full details, see the Methods section in this article's Online Repository.

**Preparation and stimulation of DNPCs**

NP tissues were harvested from patients with CRSwNP who had not taken either systemic or local glucocorticosteroids for at least 4 weeks or antibiotics for at least 2 weeks before surgery. An adequate amount of freshly obtained NP tissue was set aside for measuring cytokine levels, and the remaining NP tissue was used for DNPC preparation.

DNPCs were prepared by using enzymatic digestion, as previously described. For full details, see the Methods section in this article’s Online Repository. DNPCs were suspended in RPMI containing 10% FBS at a concentration of 2 x 10^6 cells/mL, and 0.5-mL aliquots were cultured in 24-well plates with IL-4 (50 ng/mL), IL-5 (50 ng/mL), IL-13 (50 ng/mL), IL-17 (100 ng/mL), IL-23 (100 ng/mL), TGF-β1 (100 ng/mL; R&D Systems, Minneapolis, Minn), or DEX (5 x 10^{-5} mol/L; Sigma, St Louis, Mo) for 24 hours at 37°C in a humidified incubator in a 5% CO₂ atmosphere. At the end of incubation, supernatants from each culture were collected and assayed for protein.

**Immunooassay**

Tissue homogenates, prepared as previously described, and supernatants from cultured DNPCs were assayed for levels of IL-4, IL-5, IL-13, eotaxin, IL-17, TGF-β1, IL-1β, IL-8, TNF-α, granulocyte colony-stimulating factor (G-CSF), interferon-inducible protein 10 (IP-10), myeloperoxidase (MPO), eosinophil cationic protein (ECP), and total IgE, by using commercially available kits. For full details, see the Methods section in this article’s Online Repository.

**Statistical analysis**

Relationships between TH2- and TH17-related factors were evaluated by performing a multiple correspondence analysis (MCA) and a Spearman correlation analysis. Before implementing MCA analysis, all the TH2- and TH17-related numeric variables were transformed into categorical variables. Specifically, the original numeric data were defined as "high" or "low" based on a cutoff value set according to their detection limits (for variables that had a high proportion of values under the detection limit [ie, IL-4, IL-5, and IL-17]) or their medians (for other variables). For quantitative data, the Mann-Whitney U test was used for comparing 2 groups, and the Kruskal-Wallis test with Dunn correction was used for multiple comparisons in more than 2 groups. The χ² or Fisher exact test was used for qualitative data. The Wilcoxon matched-pairs test was used to compare expression levels of patient-matched unstimulated and stimulated NP tissues. The Benjamini-Hochberg method was applied to control the false discovery rate in the multiplicity problems caused by multiple groups (ie, qualitative data) or multiple testing. P values of .05 or less were considered statistically significant. All statistical analyses were performed with IBM SPSS 20.0 software (SPSS, Chicago, Ill).

**RESULTS**

**Patients’ characteristics**

Characteristics of all participants enrolled in this study are shown in Table E1 in this article’s Online Repository at www.jacionline.org. The control and CRSwNP groups were similar with regard to age, the prevalence of comorbid asthma, and aspirin intolerance. The CRSwNP group had a significantly greater male/female ratio, comorbid atopic status, computed tomographic score, and polyp score than the control group.

**Negative interrelationships between the TH2 and TH17 inflammatory pathways in patients with CRSwNP**

IL-5 is a typical TH2 cytokine, and IL-17 is a typical TH17 cytokine. In the present study we defined the TH2 cytokines...
IL-4, IL-5, and IL-13; eosinophilic markers (eosinophil counts, ECP, and eotaxin); and total IgE as TH2-related factors and the T\(_{H}2\) cytokine IL-17; the T\(_{H}17\) differentiation cytokines IL-23, IL-1\(\beta\), and TGF-\(\beta\); the IL-17–induced cytokines IL-8, TNF-\(\alpha\), and G-CSF; and the neutrophil-related proteins IP-10 and MPO as T\(_{H}17\)-related factors.

Both IL-5 and IL-17 concentrations were significantly increased in tissue homogenates from patients with CRSwNP compared with tissue homogenates from control subjects (see Fig E1 in this article’s Online Repository at www.jacionline.org). Because we have previously shown increased expression of IL-17 in IL-5–low patients with CRSwNP, which suggests a negative association of IL-17 with IL-5, we further explored the relationships between T\(_{H}2\)- and T\(_{H}17\)-related factors in patients with CRSwNP in greater detail in this study.

MCA analysis of all patients (Fig 1) demonstrated that the low T\(_{H}2\)- and high T\(_{H}17\)-related factors were situated near each other and that the high T\(_{H}2\) variables were situated near the low T\(_{H}17\) variables. We next analyzed correlations among T\(_{H}2\)- and T\(_{H}17\)-related factors in all patients. Although most of these factors were positively correlated within the 2 sets, the 2 sets were negatively correlated (see Fig E2 in this article’s Online Repository at www.jacionline.org). These findings were consistent with MCA analysis, with both types of analyses showing negative interrelationships between the T\(_{H}2\) and T\(_{H}17\) pathways in patients with CRSwNP.

Negative interrelationships between the T\(_{H}2\) and T\(_{H}17\) pathways demonstrated above prompted us to classify our patients with CRSwNP based on distribution of the typical TH2 cytokine IL-5 and the typical T\(_{H}17\) cytokine IL-17. Four CRSwNP groups were obtained based on the detection limits of IL-5 and IL-17 used as cutoff values, including IL-5\(^+\)IL-17\(^−\), IL-5\(^−\)IL-17\(^+\), and IL-5\(^−\)IL-17\(^+\) (see Fig E3 in this article’s Online Repository at www.jacionline.org). Characteristics of these 4 CRSwNP groups are shown in Table E2 in this article’s Online Repository at www.jacionline.org and demonstrate that they were similar with regard to age, female/male ratio, computed tomographic score, and polyp score. Correlation analyses in each of the 4 CRSwNP groups showed a high number of positive correlations within the T\(_{H}2\)-related factors in the IL-5\(^+\)IL-17\(^+\) group and within the T\(_{H}17\)-related factors in the IL-5\(^−\)IL-17\(^+\) and IL-5\(^−\)IL-17\(^−\) groups but
negative correlations between those 2 sets of factors in the IL-5+IL-17− group (see Fig E4 in this article’s Online Repository at www.jacionline.org).

Expression of T\textsubscript{h}2- and T\textsubscript{h}17-related factors in the 4 CRSwNP groups defined by tissue IL-5 and IL-17 concentrations

Fig 2 shows expression levels of T\textsubscript{h}2-related factors and indicates that most of these (ie, IL-13, eotaxin, and ECP levels; ECP/MPO ratios, tissue total IgE concentrations, and blood eosinophil counts) were significantly greater in the IL-5−IL-17− group than in the 2 IL-5+ groups (IL-5+IL-17− and IL-5−IL-17−). Furthermore, tissue eosinophil counts were greater in the IL-5−IL-17− group than in the IL-5−IL-17+ group, and the ECP/MPO ratio was greater in the IL-5−IL-17− group than in the IL-5+IL-17+ group (Fig 2). Different from most T\textsubscript{h}2-related factors, IL-4 concentrations were lowest in the IL-5+IL-17− group and highest in the IL-5−IL-17+ group, with no significant difference between the IL-5−IL-17− and IL-5−IL-17+ groups (Fig 2).

In contrast to T\textsubscript{h}2-related factors, most of the T\textsubscript{h}17-related factors (ie, IL-8, IL-1β, G-CSF, and TNF-α) were expressed at significantly greater levels in the IL-5−IL-17+ group than in the 2 IL-17−negative groups (IL-5+IL-17− and IL-5−IL-17−). Furthermore, IL-8, IL-1β, G-CSF, and TNF-α levels were greater in the IL-5−IL-17+ group than in the IL-5+IL-17− group. Concentrations of MPO and IL-23 in IL-5−IL-17+ group were greater than those in the IL-5−IL-17− group but not greater than those in the IL-5+IL-17− group (Fig 3). The 4 groups showed no significant difference with regard to blood neutrophil counts (see Fig E5, A, in this article’s Online Repository at www.jacionline.org). Tissue neutrophil counts in the IL-5−IL-17− group were lower than neutrophil counts in the other 3 groups; however, no significant differences were reached (see Fig E5, B).

Associations of clinical parameters with T\textsubscript{h}2 and T\textsubscript{h}17 responses in patients with CRSwNP

Edema scores, which are used to evaluate edema formation in NPs, were positively correlated with most of the T\textsubscript{h}2-related factors (ie, IL-5 and IL-13 levels, ECP/MPO ratios, ECP levels, and blood eosinophil counts) and negatively correlated with most of the T\textsubscript{h}17-related factors (IL-17, IL-1β, TNF-α, and G-CSF levels; Fig 4, A). Similarly, edema scores in the IL-5−IL-17− group were significantly greater than in the 2 IL-5− groups (Fig 4, B). High correlation between the edema
score assessed by means of visual evaluation, and the percentage of edema area assessed by using ImageJ analysis supported the consistency of these 2 methods for evaluating edema (see Fig E6 in this article’s Online Repository at www.jacionline.org).

Collagen content was used to evaluate fibrosis in NPs and showed a strong negative correlation with edema scores \( (r = -0.752, P < .001) \). Although this finding would suggest that collagen content can be positively correlated with Th17-related factors and negatively correlated with Th2-related factors, we did not find any correlation between collagen content and Th2- or Th17-related factors, including TGF-β1. Expression of collagen in the 4 CRSwNP groups was also not significantly different among the groups (see Fig E7, A, in this article’s Online Repository at www.jacionline.org).

Frequencies of atopic status were greater in the 2 IL-5 groups than in the IL-5-IL-17 group, although they did not reach statistical significance \( (P = .101 \text{ for IL-5-IL-17}^{-}; P = .078 \text{ for IL-5+IL-17}^{-}; \text{Fig 4, C}) \). Accordingly, values of most Th2-related factors (ie, tissue IL-5 and ECP levels, total IgE concentrations, and tissue eosinophil counts) were also found to be significantly greater in allergic patients than in nonallergic patients (Fig 4, D).

The 4 groups of patients with CRSwNP showed no statistical differences with regard to the frequencies of NP recurrence (Fig 4, E), asthma occurrence, or aspirin intolerance (see Fig E7,
However, a trend toward increased frequency was noted in the IL-5⁺IL-17⁺ group \( (P = .112) \) for NP recurrence compared with the IL-5⁻IL-17⁻ group.

**Interactions of the \( \text{T}_{\text{H}}2 \) and \( \text{T}_{\text{H}}17 \) pathways in DNPCs**

Considering the negative interrelationships between \( \text{T}_{\text{H}}2 \)- and \( \text{T}_{\text{H}}17 \)-related factors in tissue from patients with CRSwNP, as demonstrated above, we hypothesized that \( \text{T}_{\text{H}}2 \) and \( \text{T}_{\text{H}}17 \) responses might interact negatively in vivo. Thus, to test this hypothesis, we investigated interactions between the \( \text{T}_{\text{H}}2 \) and \( \text{T}_{\text{H}}17 \) pathways further by using DNPCs, particularly because these presented the advantage of having all the different cell types in the polyps compared with preparations of individual cell types. In this regard all DNPCs used in the present study were from patients comprising both \( \text{T}_{\text{H}}2 \) and \( \text{T}_{\text{H}}17 \) endotypes (ie, the IL-5⁺IL-17⁺ group).

We first explored the effect of \( \text{T}_{\text{H}}2 \) cytokines on \( \text{T}_{\text{H}}17 \) response in DNPCs and found that IL-4 significantly inhibited 3 \( \text{T}_{\text{H}}17 \)-related factors (IL-17, IL-1β, and IL-8; Fig 5) and IL-13 significantly inhibited 6 \( \text{T}_{\text{H}}17 \)-related factors (IL-1β, IP-10, TNF-α, MPO, IL-8, and G-CSF; Fig 6). In contrast, no \( \text{T}_{\text{H}}17 \)-related factor in DNPCs was significantly inhibited by IL-5 stimulation (see Fig E8 in this article’s Online Repository at www.jacionline.org).

We next explored the effects of \( \text{T}_{\text{H}}17 \) cytokine stimulation on the \( \text{T}_{\text{H}}2 \) response in DNPCs and found that IL-4 significantly inhibited 3 \( \text{T}_{\text{H}}17 \)-related factors (IL-17, IL-1β, and IL-8; Fig 5) and IL-13 significantly inhibited 6 \( \text{T}_{\text{H}}17 \)-related factors (IL-1β, IP-10, TNF-α, MPO, IL-8, and G-CSF; Fig 6). In contrast, no \( \text{T}_{\text{H}}17 \)-related factor in DNPCs was significantly inhibited by IL-5 stimulation (see Fig E8 in this article’s Online Repository at www.jacionline.org).

**FIG 5.** Inhibitory effect of IL-4 stimulation on \( \text{T}_{\text{H}}17 \) cytokines in DNPCs: IL-4, 50 ng/mL (n = 10). The statistical significance of differences in expression was assessed by using the Wilcoxon matched-pairs test.
Inhibition of both T_{H2} and T_{H17} responses in DNPCs by using DEX treatment

Given the wide use of corticosteroids for patients with CRSwNP, we investigated the effect of DEX on T_{H2} and T_{H17} responses in DNPCs and found that treatment with DEX significantly decreased concentrations of 3 T_{H2}-related cytokines (IL-4, IL-5, and eotaxin) and concentrations of 6 T_{H17}-related cytokines (IL-17, IL-1β, TNF-α, MPO, IL-8, and G-CSF) in DNPCs (see Fig E10 in this article’s Online Repository at www.jacionline.org).

**DISCUSSION**

Increasing numbers of studies have revealed that CRSwNP is a heterogeneous disease consisting of many endotypes driven by distinct molecular mechanisms.\[3,4,10\] Using a multicenter study design and the same Chinese subjects as in the present study, we have previously shown that the T_{H17} pathway, in addition to the T_{H2} pathway, contributed to the inflammatory process involved in patients with CRSwNP, especially in mainland China. This study further demonstrated that polyps in patients from Asia were clearly different from those in Europe, with significantly more T_{H2} expression present in polyps from European patients.\[2\] However, it remains unclear how the T_{H2} and T_{H17} pathways interact in patients with CRSwNP and how the observed difference between polyps in Asia and Europe might develop.

In the present study the negative interrelationship between T_{H2}- and T_{H17}-related factors demonstrated by using MCA and correlation analysis and the exclusive expression of these 2 kinds of factors in 2 single-positive groups suggest a mutual exclusion of the T_{H2} and T_{H17} pathways. These observations can be extended to asthma. The intercorrelations between T_{H2} and
TH17 cytokines were previously explored in NPs from patients with and without cystic fibrosis in Belgium and did not demonstrate similar findings to ours; however, cystic fibrosis is a genetically determined disease different from adult NPs. The less pronounced dominance of the TH2 pathway in China compared with Europe makes it preferable to study the TH2 and TH17 pathway interrelationship in China rather than in Europe. The factors possibly contributing to the TH17 expression in China have been discussed in detail in a previous study.

The lack of relationships of TGF-β1 with TH17- and TH2-related factors observed in our study might be explained by the fact that TGF-β1, although a TH17-related factor, was also produced prominently by eosinophils.

As expected, stimulation of DNPCs in vitro with the TH2 cytokines IL-4 and IL-13 inhibited TH17 cytokine production. However, IL-5 had no obvious effect in this regard, despite the negative correlation between IL-5 and IL-17 levels in patients with CRSwNP. Conversely, stimulation with the TH17 cytokines IL-17 and TGF-β1, but not IL-23, enhanced TH2 cytokine production. This suppressive effect of TH17 cytokines on TH17 cytokines in DNPCs is consistent with previous studies conducted in murine asthma models, atopic dermatitis models, epithelial cells, and in vitro studies of TH17 cell differentiation.

Effects of TH17 cytokines on the TH2 response varied and appeared to be complicated, such as the conflicting regulation of IL-17 on TH2 responses in murine models of lung disease and in vitro culture of epithelial cells and fibroblasts, inhibition of TH2 differentiation by TGF-β1, increased eotaxin production in airway fibroblasts by TGF-β1 plus IL-13 stimulation, and upregulation of TH2 cell–mediated eosinophilic airway inflammation by IL-23 in mice. Despite this, our findings support a positive regulation of the TH2 response by TH17 cytokines in Chinese patients with CRSwNP.

Because type 17 immune markers enhance the expression of type 2 immune markers, immune reactions can change the expression pattern from the IL-5−IL-17+ group to the IL-5+IL-17+ group, stabilizing the new constellation with a high number of positive correlations. Both sides of the interaction that occurs between the TH2 and TH17 pathways favor development of the TH2 immune response. Because environmental factors, such as allergen exposure and Staphylococcus aureus colonization, have been reported to induce TH2 responses, the increased TH2 response in allergic patients with CRSwNP compared with that in nonallergic patients with CRSwNP in our study supports a role of allergy and allergen exposure for the TH2 response in a situation with rather modest TH2 immune responses. In contrast, it appears that S aureus does not play a role in stimulation of a TH2 response because only 3 of the 95 Chinese CRSwNP patients in our study were S aureus enterotoxin-specific IgE positive (data not shown). S aureus enterotoxin-specific IgE positivity in European patients with CRSwNP is associated with a much stronger type 2 immune reaction. Therefore complex regulation of the TH2 and TH17 pathways and allergen exposure might both contribute to the overriding TH2 inflammation seen in patients with CRSwNP in China, with 23% and 38% of patients with CRSwNP belonging to the IL-5−IL-17+ and IL-5+IL-17+ groups, respectively. Moreover, this might provide an explanation for the possible difference toward type 2 CRSwNP endotypes from Asia to Europe and the recently described shift in Asia, as indicated by the increased eosinophilic signature of CRSwNP in Thailand and Korea over time.

Unlike the strong correlation of tissue IL-5 levels with tissue and blood eosinophil numbers, tissue IL-17 levels were strongly correlated with tissue MPO levels but not tissue or blood neutrophil numbers (data not shown). This provided direct evidence that neutrophil activity might be more important than...
neutrophil numbers in TH17 inflammation, as has been assumed in a study using a mouse model of allergen-induced asthma. Complex regulation of the TH2 and TH17 pathways in patients with CRSwNP also has important implications for CRSwNP treatments. Several TH2-targeted therapies, such as anti-IL-5, anti-IL-4, and anti-IL-13, have been developed for patients with CRSwNP. Inhibitory effects of IL-4 and IL-13 on the TH17 response in DNPCs and the increased TH17 response produced by anti-IL-4/IL-13 treatments in an asthma mouse model demonstrate the need to monitor whether long-term anti-IL-4/IL-13 therapies will produce adverse side effects by increasing the TH17 response in patients with CRSwNP. Our results would not point in that direction. Furthermore, involvement of a TH17 response and the complex regulation of the TH2 and TH17 pathways in patients with CRSwNP might necessitate the need to treat the disease by targeting TH2-related factors and TH17-related targets.

Topical and systemic corticosteroids are generally used as first-line therapy for NPs. The decreased IL-4, IL-5, and eotaxin concentrations in DNPCs treated with DEX in our study confirmed the inhibitory effect of corticosteroids on the TH2 response. Results of previous studies in patients and mice suggest that this type of TH2 inhibition caused by corticosteroids might subsequently cause an increased TH17 response caused by loss of TH2 cytokine–induced inhibition. However, decreased concentrations of TH17-related cytokines in DNPCs treated with DEX in our study suggests that DEX suppressed, rather than enhanced, the TH17 response in DNPCs, which is in accordance with results of studies conducted using NP explants and in psoriatic skin. The direct inhibitory effect of a corticosteroid on the TH17 response might override the loss of inhibition by TH2 cytokines after corticosteroid treatment.

Edema formation in NPs has previously been reported to be associated with eosinophil infiltration. In this regard our study has shown a positive correlation between edema scores and TH2-related factors and a negative correlation between edema scores and TH17-related factors, suggesting that the TH2 pathway promotes edema formation, whereas the TH17 pathway inhibits edema formation. Our finding that edema scores in the IL-5+IL-17− group were greater than those in the IL-5 IL-17+ group further supports this hypothesis. Despite the negative correlation between collagen (a marker of fibrosis) and CRSwNP edema scores in our study, no expected negative correlation between collagen and the TH2 pathway or positive correlation between collagen and the TH17 pathway was seen. This could be explained by evidence showing that both IL-17a and IL-13 might be associated with fibrosis.

There are several limitations to this study. First, we did not assess the longitudinal intraintividual changes in TH2 and TH17 responses, and thus no direct evidence in patients was provided to support the assumption that the interactions between the TH2 and TH17 pathways ultimately lead to a TH2 shift. Second, IL-5 and IL-17 concentration measurement in NPs is dependent on performing invasive procedures, and this limits the routine use of IL-15 and IL-17 as biomarkers for TH2 and TH17 pathway activities, respectively. Third, the DNPC system used in this study is comprised of a wide variety of cell types rather than a specific cell type, and this made it impossible to explain the specific mechanisms at cellular level. Furthermore, the balance of cell populations in the DNPC system might be altered because of the cells dying during culture, which might affect the results.

In conclusion, our data show that the TH2 and TH17 pathways in Chinese patients with CRSwNP are mutually exclusive, and the interaction of these 2 pathways favors development of a TH2 response. Atopy might play a further role in the overriding TH2 response in patients with CRSwNP in China. The TH2 and TH17 pathways can both be inhibited by DEX and might inversely affect edema formation, with the TH2 pathway being predominate. Overall, this study provides useful insight into a currently observed shift toward type 2 endotypes in patients with CRSwNP in Asia over time and from Asia to Europe, as well as endotype classification and treatment criteria for CRSwNP.

**Clinical implications:** This study adds new information on the molecular mechanisms driving distinct endotypes of CRSwNP by showing the complex regulation of TH2 and TH17 immunity in patients with CRSwNP, possibly explaining the type 2 shift observed in Asia over time.

**REFERENCES**


