Nasal IL-4+CXCR5+CD4+ T follicular helper cell counts correlate with local IgE production in eosinophilic nasal polyps

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Background: Locally produced IgE contributes to the initiation and development of eosinophilic inflammation in eosinophilic nasal polyps independent of systemic atopy. However, whether CXCR5+CD4+ T follicular helper (T\textsubscript{FH}) cells are involved in local IgE production at mucosal sites remains unexplored. Objective: We sought to explore the presence, phenotype, and function of CXCR5+CD4+ T\textsubscript{FH} cells in eosinophilic nasal polyp tissues compared with noneosinophilic nasal polyp and control normal nasal tissues. Methods: T\textsubscript{FH} cell-surface phenotypes and subsets and B-cell subsets in nasal tissues and peripheral blood were studied by means of flow cytometry. Immunohistochemistry was used to detect the tissue location of T\textsubscript{FH} cells. Sorted nasal T\textsubscript{FH} cells and CXCR5+ T cells were cultured with autologous naive B cells purified from blood. Results: Nasal T\textsubscript{FH} cells expressed inducible costimulator, programmed cell death protein 1, and the transcription factor B-cell lymphoma 6 at an intermediate level when compared with bona fide T\textsubscript{FH} cells in tonsils and circulating T\textsubscript{FH} cells. Although counts of total T\textsubscript{FH} cells and IL-21+, IFN-\gamma+, and IL-17+ T\textsubscript{FH} cells were increased in both eosinophilic and noneosinophilic nasal polyp tissues compared with those in normal nasal tissues, IL-4+ T\textsubscript{FH} cell counts were only increased in eosinophilic polyp tissues. IL-4 and IL-21 were involved in polyp T\textsubscript{FH} cell–induced IgE production from naive B cells, and nasal IL-4+ T\textsubscript{FH} cell counts correlated highly with local IgE levels in vivo. IL-4+Bcl-6+CD4+ T\textsubscript{FH} cells were identified in ectopic lymphoid structures in eosinophilic nasal polyps. T\textsubscript{FH} cells also positively correlated with germinal center B cells and plasma cells in nasal tissues. Conclusion: Nasal IL-4+ T\textsubscript{FH} cells might be involved in local IgE production in eosinophilic nasal polyps. (J Allergy Clin Immunol 2016;137:462-73.)

Key words: B cell, ectopic lymphoid structure, eosinophil, IgE, IL-4, nasal polyp, T follicular helper cell

Despite advances in medical and surgical therapy, chronic rhinosinusitis remains difficult to treat, particularly for patients with chronic rhinosinusitis with nasal polyps (CRSwNP). A great obstacle in improving the treatment of chronic rhinosinusitis is our limited understanding of the mechanisms of this complex and heterogeneous disease. Eosinophilic inflammation has commonly been considered a cardinal feature of CRSwNP in white subjects. In Asian subjects half of CRSwNP cases also present with eosinophilic inflammation. The ultimate factors in inducing this mucosal eosinophilia remain uncertain; however, increased local IgE production in polyp tissues might contribute to mucosal mast cell activation and eosinophilic inflammation independent of systemic atopy. Although B-cell class-switch recombination (CSR) to IgE has been generally assumed to be restricted to the germinal centers (GCs) of lymphoid organs, the presence of follicle-like structures and the expression of CSR markers, including e germline gene transcript and e circle transcripts in polyp tissues, strongly suggest local CSR to IgE in patients with eosinophilic CRSwNP.1,6

The B-lymphocyte CSR to IgE is initiated by the cytokines IL-4 or IL-13, which have long been believed to be produced by T\textsubscript{H2} cells. Recently, it has become clear that a distinct subset of T\textsubscript{H2} cells beyond the T\textsubscript{H1}/T\textsubscript{H2} paradigm, termed T follicular helper (T\textsubscript{FH}) cells largely on the basis of their localization in B-cell follicles, plays a crucial role in B-cell response induction. T\textsubscript{FH} cells are also distinguishable from other T\textsubscript{H2} cells by increased expression of CXCR5, inducible costimulator (ICOS), and programmed cell death protein 1 (PD1) and the transcription factor B-cell lymphoma 6 (Bcl-6) and v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog (c-Maf); downregulation of CCR7, CD127, and B lymphocyte–induced maturation protein 1 (Blimp1); and production of the canonical cytokine IL-21.7,8 The fundamental role of T\textsubscript{FH} cells...
in humoral immunity has resulted in many studies designed to understand their roles in, for example, human infections, autoimmune diseases, and vaccination.\textsuperscript{9,10} In addition to secondary lymphoid organs, human CXCR5\textsuperscript{+}CD4\textsuperscript{+}TFH cells have also been identified in peripheral blood, sharing similar functional properties with \textit{bona fide} GC TFH cells in secondary lymphoid organs and possibly representing a circulating memory compartment of TFH lineage cells.\textsuperscript{11-15} Recently, the presence of CXCR5\textsuperscript{+}CD4\textsuperscript{+}TFH cells has also been documented in ectopic lymphoid structures in lesional tissues, such as breast cancer and rheumatoid arthritis synovium\textsuperscript{16,17}; nevertheless, the phenotypes and functions of these CXCR5\textsuperscript{+}CD4\textsuperscript{+}TFH cells in nonlymphoid lesional tissues have yet to be defined.

Given the local CSR to IgE in patients with eosinophilic CRSwNP and the pivotal role of TFH cells in immunoglobulin production, we hypothesized that CXCR5\textsuperscript{+}CD4\textsuperscript{+}TFH cells might be present in nasal polyp tissues and involved in local IgE production. In this study we comprehensively evaluated nasal mucosal CXCR5\textsuperscript{+}CD4\textsuperscript{+}TFH cell numbers, phenotype, and function in patients with eosinophilic and noneosinophilic CRSwNP. We reported that increased nasal mucosal IL-4\textsuperscript{+}CXCR5\textsuperscript{+}CD4\textsuperscript{+}TFH cell counts correlate with local IgE production in eosinophilic polyps. Nasal mucosal CXCR5\textsuperscript{+}CD4\textsuperscript{+}TFH cells manifest different phenotypic characteristics compared with circulating and \textit{bona fide} TFH cells.

**METHODS**

**Patient population and clinical samples**

This study was approved by the Ethics Committee of Tongji Hospital and conducted with written informed consent from each patient. The diagnosis of CRSwNP was made according to the current European Academy of Allergy and Clinical Immunology “European position paper on rhinosinusitis and nasal polyps 2012.”\textsuperscript{21} CRSwNP was defined as eosinophilic when the percentage of tissue eosinophils exceeded 10\% of total infiltrating cells, as reported by our previous study.\textsuperscript{2} Subjects undergoing septoplasty because of percentage of tissue eosinophils exceeded 10\% of total infiltrating cells, as previously described.\textsuperscript{18} Consecutive sections were stained to assess significant intergroup variability. The 2-tailed Mann-Whitney \textit{H} test was used for between-group comparison. The Spearman rank test was used for correlations. Significance was accepted at a \textit{P} value of less than .05. For multiple comparisons among 3 study groups, Bonferroni correction was used to adjust the significance level by using an \textit{α} value of \(0.05/3 = 0.017\) for each comparison.

**Histologic study**

Hematoxylin and eosin and immunohistochemical staining were conducted, as previously described.\textsuperscript{2} Consecutive sections were stained to study the relationship between CD4, IL-4, and Bcl-6 expression. Antibodies used are listed in Table E2 and other additional information is provided in the Methods section in this article’s Online Repository.

**Isolation and purification of naive B cells and TFH cells**

Naive CD19\textsuperscript{+}IgD\textsuperscript{+} B cells, CXCR5\textsuperscript{+}CD4\textsuperscript{+} T cells, and CXCR5\textsuperscript{+}CD4\textsuperscript{+} TFH cells were isolated by means of immunomagnetic cell sorting from peripheral blood and nasal polyp tissues, respectively.\textsuperscript{18} The representative results of purification of B and T cells are shown in Fig E1 and more information is provided in the Methods section in this article’s Online Repository.

**Coculture of CD4\textsuperscript{+} T cells and autologous naive B cells**

Sorted TFH cells or CXCR5\textsuperscript{+} T cells (30 \(\times\) 10\textsuperscript{3} cells per well) were cocultured with autologous naive B cells (25 \(\times\) 10\textsuperscript{3} cells per well) for 8 days in U-bottom, 96-well plates, as previously described.\textsuperscript{19} More information is provided in the Methods section in this article’s Online Repository.

**Immunoglobulin measurement**

Protein levels of immunoglobulins in tissue homogenates and cell-culture supernatants were detected by using Bio-Plex suspension chip technology (Bio-Rad Laboratories, Hercules, Calif).\textsuperscript{18} Total IgG was calculated as the sum of the 4 subclasses, as previously mentioned.\textsuperscript{1} Specific IgE to Der p 1 was detected by using the ImmunoCAP system (Phadia, Uppsala, Sweden).\textsuperscript{5} Detection limit for Bio-Plex assay is listed in Table E4 and other additional information is provided in the Methods section in this article’s Online Repository.

**Quantitative real-time PCR**

Quantitative RT-PCR was performed with specific primers, as stated elsewhere.\textsuperscript{2,24} More information is provided in the Methods section and Table E5 in this article’s Online Repository.

**Statistics**

Statistical analysis was performed with SPSS 13.0 software (SPSS, Chicago, Ill). Expression data are presented in dot plots. Symbols represent individual samples; horizontal bars represent medians, and error bars show interquartile ranges. Cell-culture data are expressed as means ± SDs. When comparisons were made between groups, the Kruskal-Wallis \textit{H} test was used to assess significant intergroup variability. The 2-tailed Mann-Whitney \textit{U} test was used for between-group comparison. The Spearman rank test was used for correlations. Significance was accepted at a \textit{P} value of less than .05. For multiple comparisons among 3 study groups, Bonferroni correction was used to adjust the significance level by using an \textit{α} value of \(0.05/3 = 0.017\) for each comparison.
RESULTS
Enhanced local immunoglobulin levels in nasal polyp tissues

We first confirmed increased local IgE levels in eosinophilic polyps in comparison with that seen in noneosinophilic polyps and control nasal tissues (Fig 1). Moreover, we found that levels of total IgG, IgG1, IgG2, IgG4, and IgA in nasal tissues were upregulated similarly in both patients with eosinophilic and those with noneosinophilic CRSwNP compared with those in control subjects (Fig 1).

Enrichment of CXCR5+CD4+ TFH cells in nasal polyp tissues

Given the enhanced local immunoglobulin levels in nasal polyps, we next explored the presence of TFH cells in nasal polyps. As shown in Fig E2 in this article’s Online Repository at www.jacionline.org, the mRNA expression of the TFH cell transcription factor genes B-cell lymphoma 6 (BCL6) and v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog and a transcription factor gene critical for plasma cell differentiation, B lymphocyte–induced maturation protein 1 (BLIMP1), was increased in both eosinophilic and noneosinophilic polyps compared with that seen in control nasal tissues. CD40 ligand and neuropilin 1 (Nrp1) are the cell-surface molecules expressed on activated TFH cells. The mRNA expression of CD40L and neuropilin 1 (NRP1) has been found to be upregulated in eosinophilic and noneosinophilic polyps. In addition, the mRNA expression of IL-21 and IL-21 receptor (IL21R), which are critical in regulating TFH cell and B-cell differentiation, were markedly enhanced in polyp tissues. There was also a significant upregulation of the expression of B-cell and TFH cell chemokines and corresponding chemokine receptor genes in polyps, including CXCL13-CXCR5 and CXCL12-CXCR4, compared with that seen in control nasal tissues.

Our previous study demonstrated a similar increase in NMC counts in eosinophilic and noneosinophilic polyps compared with those in control nasal tissues.2 Current flow cytometric analysis revealed a higher frequency of CD4+ T cells within NMCs from both patients with eosinophilic and those with noneosinophilic CRSwNP compared with that seen in control subjects (Fig 2, B). Importantly, for the first time, we discovered that the frequencies of CXCR5+CD4+ TFH cells in both NMCs and CD4+ T cells were increased similarly in eosinophilic and noneosinophilic polyp tissues when compared with those in control tissue (Fig 2, B). Further analysis revealed that these nasal mucosal CXCR5+CD4+ TFH cells can also express ICOS and PD1. The percentages of ICOS+, PD1+, and ICOS+PD1+ TFH cells among NMCs and total CXCR5+CD4+ TFH cells were higher either in both eosinophilic and noneosinophilic polyps or only in eosinophilic polyps than in control tissues (Fig 2, C and D). In contrast, in peripheral blood no significant difference in the frequencies of the major types of TFH cells could be found among different study groups (see Fig E3 in this article’s Online Repository at www.jacionline.org). Representative fluorescence-activated cell sorting (FACS) plots showing the frequencies of TFH cells in tissue and blood in different study groups are shown in Figs E4 and E5, respectively, in this article’s Online Repository at www.jacionline.org.

Increased IL-4+ TFH cell subsets in eosinophilic nasal polyp tissues

Despite enhanced local IgE production in patients with eosinophilic CRSwNP compared with that seen in patients with noneosinophilic CRSwNP, we did not find a significant difference in total, ICOS+, and PD1+ TFH cell counts between eosinophilic and noneosinophilic polyps. This prompted us to analyze the polarization of TFH cells toward Th1, Th2, and Th17 phenotypes in nasal polyp tissues. First, we found that the frequencies of IL-21+, IFN-γ+, and IL-17A+ cells within the NMCs were increased in both eosinophilic and noneosinophilic polyps compared with control tissues; however, the percentages of IL-4+ TFH cells were significantly higher in eosinophilic polyps compared with noneosinophilic polyps.
and IL-4+IL-21+ cells within NMCs were only increased in eosinophilic polyps compared with those in noneosinophilic polyps and control tissues (Fig 3, B). Furthermore, we discovered that the percentages of IL-21+, IFN-γ+, and IL-17A+ TFH cells in both NMCs and total CXCR5+CD4+ TFH cells were increased similarly in eosinophilic and noneosinophilic polyps compared with those of control tissue, although the percentages of IL-4+ and IL-4+IL-21+ TFH cells among both NMCs and total TFH cells were only increased in eosinophilic polyps in comparison with those in noneosinophilic polyps and control tissues (Fig 3, C and D).

We next assessed whether the increase in TFH cell counts in eosinophilic polyp tissues might also be reflected in an antigen-specific expansion or was independent of antigen specificity. Our previous study has shown that dust mite allergens are one of the major antigens driving local IgE production in Chinese patients with eosinophilic CRSwNP. Therefore we selected eosinophilic polyps with local IgE against Dermatophagoides pteronyssinus group 1 (Der p 1) allergen and compared them with noneosinophilic polyps without obvious local IgE against Der p 1 (see Table E6 in this article’s Online Repository at

FIG 2. Expansion of CXCR5+CD4+ TFH cells in nasal polyp tissues. A, Gating strategy and representative flow plots. TFH cells were defined as CXCR5+CD4+ and further characterized based on ICOS and PD1 expression. B, Frequencies of CD4+ T cells and TFH cells in NMCs and percentages of TFH cells in total CD4+ T cells. C and D, Frequencies of ICOS+, PD1+, and ICOS+PD1+ TFH cells in NMCs (Fig 2, C) and total TFH cells (Fig 2, D). Eos CRSwNP, Eosinophilic CRSwNP; Non-Eos CRSwNP, noneosinophilic CRSwNP.
We stimulated NMCs extracted from polyp tissues with Der p 1 allergen. We found that the percentages of IL-4, IL-21, and IL-41IL-21 TFH cells were significantly increased in patients with eosinophilic CRSwNP but not in patients with noneosinophilic CRSwNP after stimulation (see Fig E6 in this article’s Online Repository at www.jacionline.org), indicating an antigen-specific TFH cell response in patients with eosinophilic CRSwNP.

We also investigated the distribution of nasal mucosal TFH cells using immunohistochemical staining. Consistent with our previous report, a lymphoid follicle-like structure with T/B-cell aggregation could be found in both eosinophilic and noneosinophilic polyp tissues. Bcl-6+CD4+ TFH cells could be identified in these ectopic lymphoid structures in both eosinophilic and noneosinophilic polyps, whereas IL-4+ Bcl-6+CD4+ TFH cells were mainly found in ectopic lymphoid structures in eosinophilic polyp tissues (Fig 4).

Again, there was no significant difference in the percentage of IL-21+, IL-4+, IFN-γ, IL-17A+ or IL-21+IL-4+ cells or the corresponding TFH cell subset in the blood among different study groups (see Fig E7 in this article’s Online Repository at www.jacionline.org). Representative FACS plots showing the frequencies of these cells in tissue and blood in different study groups are shown in Figs E8 and E9, respectively, in this article’s Online Repository at www.jacionline.org.

Distinct phenotype of nasal polyp CXCR5+CD4+ TFH cells

Previous studies demonstrated a distinct phenotype of blood CXCR5+CD4+ TFH cells compared with bona fide GC TFH cells.13,15 Consistent with those previous reports,13,15 no obvious Bcl-6 expression and low intensity of ICOS and PD1 expression were found in blood TFH cells in this study (Fig 5).
FIG 4. Distribution of IL-4+ TFH cells in ectopic lymphoid structures in eosinophilic nasal polyp tissues. A and B, Representative photomicrographs showing successive serial sections of paraffin-embedded nasal polyp tissue from a patient with eosinophilic nasal polyps stained by using immunohistochemistry for CD20, CD4, IL-4, and Bcl-6. Fig 4, A, Original magnification x200. Fig 4, B, Original magnification x400. C, Higher magnification of the outlined area of Fig 4, B. Arrows of the same color indicate the same cell in serial sections.

FIG 5. Phenotypic characteristics of nasal polyp (NP) CXCR5+CD4+ TFH cells. Compared with those of tonsillar and circulating TFH cells, the frequencies of Bcl-6+, IL-21+, ICOS+, PD1+, and ICOS+PD1+ TFH cells within total CXCR5+CD4+ TFH cells were presented at intermediate levels in eosinophilic and noneosinophilic nasal polyp tissues. Similar to tonsillar and circulating TFH cells, most nasal polyp TFH cells were CD45RA- memory cells.
and see Fig E10 in this article’s Online Repository at www.jacionline.org). Similar to bona fide GC T\textsubscript{FH} cells in tonsils, circulating T\textsubscript{FH} cells in blood, or both, we found that most nasal polyph T\textsubscript{FH} cells were CD45RA\textsuperscript{−} memory cells and expressed higher levels of ICOS and Bcl-6 than CXCR5\textsuperscript{−} T cells (see Fig E10, A). Moreover, we found that polyph T\textsubscript{FH} cells expressed T\textsubscript{FH} cell markers, including Bcl-6, IL-21, ICOS, and PD1, at an intermediate level compared with tonsillar GC T\textsubscript{FH} cells and circulating T\textsubscript{FH} cells (Fig 5). In addition, we found that polyph T\textsubscript{FH} cells produced more IL-21 and IL-4 than CXCR5 T cells (see Fig E10, B). In contrast, polyph CXCR5 T cells expressed higher levels of IFN-\textgamma and IL-17A than T\textsubscript{FH} cells (see Fig E10, B). Representative FACS plots showing expression of Bcl-6, IL-21, ICOS, PD1, and CD45RA in CXCR5\textsuperscript{−}CD4\textsuperscript{+} and CXCR5\textsuperscript{−}CD4\textsuperscript{+} cells from tonsils, nasal polyps, and blood are shown in Fig E11 in this article’s Online Repository at www.jacionline.org.

**Nasal polyph IL-4\textsuperscript{+} T\textsubscript{FH} cells participate in IgE production**

Sequentially, we investigated the helper function of nasal polyph CXCR5\textsuperscript{−}CD4\textsuperscript{+} T\textsubscript{FH} cells on naive B cells. We found that polyph CXCR5\textsuperscript{−} T\textsubscript{FH} cells presented potent capacity to induce IgM, IgG1, IgG3, IgG4, IgA, and IgE production, whereas polyph CXCR5\textsuperscript{−} T cells induced production of these immunoglobulins at very low levels (Fig 6, A). Consistent with previous reports,\textsuperscript{11} we did not find a significant difference in T-cell viability between CXCR5\textsuperscript{−} and CXCR5\textsuperscript{−} T cells after coculture (data not shown), suggesting that the inability of CXCR5\textsuperscript{−} T cells to induce immunoglobulin production was not caused by their survival status. In coculture of CXCR5\textsuperscript{−}CD4\textsuperscript{+} T\textsubscript{FH} cells sorted from eosinophilic polyps with naive B cells, IL-21 blockade resulted in decreased IgG, IgA, and IgE production (Fig 6, B), suggesting that similar to circulating and GC T\textsubscript{FH} cells, the capacity of nasal polyph CXCR5\textsuperscript{−}CD4\textsuperscript{+} T\textsubscript{FH} cells to promote immunoglobulin production is dependent to some extent on IL-21. However, blocking IL-4 resulted in a substantial inhibition of IgE production but not of other immunoglobulins (Fig 6, B). In a blocking experiment IgG1 or IgG2 could not be detected because of cross-reactivity with blocking experiment reagents.

**Nasal IL-4\textsuperscript{+} T\textsubscript{FH} cell counts correlate with local IgE levels**

We analyzed the relationship between different T\textsubscript{FH} cell subsets and local immunoglobulin levels in nasal mucosa. We discovered that the frequencies of local IL-4\textsuperscript{+}, IL-21\textsuperscript{+}, and IL-4\textsuperscript{+}IL-21\textsuperscript{+} T\textsubscript{FH} cells, but not total T\textsubscript{FH} cells, IFN-\textgamma\textsuperscript{+} T\textsubscript{FH} cells, or IL-17A\textsuperscript{+} T\textsubscript{FH} cells, were positively correlated with IgE levels in nasal tissues (Fig 8). In addition, percentages of IL-21\textsuperscript{+} and IL-17A\textsuperscript{+} T\textsubscript{FH} cells were positively correlated with IgG levels (see Fig E13 in this article’s Online Repository at www.jacionline.org), and percentages of total T\textsubscript{FH} cells and IL-4\textsuperscript{−}, IL-21\textsuperscript{−}, and IL-4\textsuperscript{−}IL-21\textsuperscript{−} T\textsubscript{FH} cells were positively correlated with IgA levels in nasal tissues (Fig 8 and see Fig E13). No significant correlation between total T\textsubscript{FH} cells or T\textsubscript{FH} cell subsets and IgM levels in nasal tissue was discovered (Fig 8 and see Fig E13).

**DISCUSSION**

Local IgE production might play an important role in the development of mucosal eosinophilia not only in nasal polyps but also in patients with allergic rhinitis, asthma, and eosinophilic esophagitis, yet the mechanisms underlying this mucosal IgE overproduction remain poorly understood.\textsuperscript{3,21,22} Recently, the
FIG 7. Skewing within the B-cell compartment in nasal polyp tissues correlates with local CXCR5⁺ CD4⁺ TFH cell expansion. A, Gating strategy and representative flow plots. B cells were defined as CD3⁻ CD19⁺ and further characterized based on the Bm classification: Bm1 (naive), CD38⁻ IgD⁺; Bm2 (activated naive), CD38⁺ IgD⁻; Bm29 (pre-GC), CD38⁻ IgD⁻; Bm3/4 (GC), CD38⁺ IgD⁺; Early Bm5 (early memory), CD38⁻ IgD⁻; Late Bm5 (late memory), CD38⁺ IgD⁺; and plasma cells (PCs), CD38⁺⁺ IgD⁻. B, Frequencies of different B-cell compartments within total B cells in nasal tissues from control subjects, patients with eosinophilic CRSwNP (Eos CRSwNP), and patients with noneosinophilic CRSwNP (Non-Eos CRSwNP). C, Significant positive correlations between TFH cell frequency and GC (Bm3/4) B-cell and PC frequency and a significant negative correlation between TFH cell frequency and naive (Bm1) B-cell frequency in the sinonasal mucosa from all study groups (n = 30).
percentages of circulating TFH cells have been found to be increased in patients with autoimmune diseases and correlated with serum concentrations of IgG autoantibodies, however, the role of TFH cells in patients with IgE-mediated diseases has been barely studied.

In the present study we provided evidence of accumulation and expansion of CXCR5+CD4+ TFH cells in both eosinophilic and noneosinophilic nasal polyp tissues. These mucosal TFH cells demonstrated phenotypic differences compared with bona fide GC TFH cells and blood TFH cells, with the expression of ICOS, PD1, and Bcl-6 at an intermediate level. These TFH cells could be identified in ectopic lymphoid structures in nasal polyp tissues and could efficiently induce immunoglobulin production from naive B cells. These features of nasal TFH cells are partly similar to those of CXCR5ICOSPD1+ T cells residing outside of GCs, which are possible precursors of GC TFH cells in human tonsils, indicating an immature phenotype of TFH cells in ectopic lymphoid structures. To clarify the ontogeny of these human TFH cells, we found here that nasal IL-4+ TFH cells were increased comparably in eosinophilic and noneosinophilic nasal polyps, further suggesting a dysfunction of TFH cells in diseased mucosa of patients with CRSwNP.

Recent studies have demonstrated that blood memory TFH cells are composed of heterogeneous cell populations with discrete capabilities to support B cells to produce antibodies. CXCR3+CCR6+ (Th12) and CXCR3+CCR6+ (Th17) TFH cells can induce B cells to produce IgG and IgE and IgG and IgA, respectively, whereas CXCR3+CCR6+ (Th11) TFH cells do not produce IL-21 and lack the ability to help naive B cells. The enhanced local IgE production in eosinophilic but not noneosinophilic nasal polyps prompted us to explore whether polyp TFH cells encompass distinct subsets and whether the skewing of polyp TFH cell subsets might underlie the IgE overproduction in eosinophilic polyps. We found that the frequency and numbers of IL-21+, IL-17+, and IFN-γ+ TFH cells were increased comparably in eosinophilic and noneosinophilic polyps; in contrast, IL-4+ and IL-4+IL-21+ TFH cells were uniquely increased in eosinophilic polyps, depicting distinct profiles of TFH cell subsets in nasal polyps with different inflammation patterns. We also tested the relationship between CXCR3+CCR6+ TFH cells and IL-4+ TFH cells in nasal polyps. Consistent with the findings in circulating CXCR5+CD4+ TFH cells, we found here that nasal IL-4+ TFH cells were not definitely CXCR3+CCR6+ cells because some of them could be found in CXCR3CCR6+ and CXCR3+CCR6+ TFH cells, and CXCR3+CCR6+ TFH cells were not necessarily IL-4+ cells (data was not shown). Therefore given the importance of the cytokines in inducing immunoglobin class-switching, we believe that they might be more suitable to define the TFH cell subset based on cytokine expression in our current study. Importantly, we revealed that only CXCR5+CD4+ TFH cells from eosinophilic polyps, but not those from noneosinophilic polyps, promoted IgE production, which could be suppressed by IL-21 and IL-4 blockade. Moreover, we found that the frequency of IL-4+ cells was higher in CXCR5+ TFH cells than in CXCR5+ T cells in nasal polyp tissues and that IL-4+Bcl-6+CD4+ TFH cells existed in ectopic lymphoid structure in eosinophilic polyps. These results underscore a critical role of nasal mucosal IL-4+ TFH cells in local IgE induction, which was further confirmed in vivo by the
finding that the number of nasal mucosal IL-4+, IL-21+, and IL-4+/IL-21+ Tfh cells, but not total CXCR5+CD4+ Tfh cells or other Tfh cell subsets, correlated with local IgE levels. Our previous study has shown that dust mite allergens are the major antigens driving local IgE production in Chinese patients with eosinophilic CRSwNP. Here we demonstrated the presence of dust mite–specific Tfh cells in eosinophilic polypos with local IgE against dust mites, reflecting an antigen-specific expansion of lesional IL-4+ Tfh cells in patients with eosinophilic CRSwNP. In contrast to IgE, we found that IgG and IgA levels were similarly enhanced in eosinophilic and noneosinophilic polypos and that CXCR5+CD4+ Tfh cells from eosinophilic and noneosinophilic polypos induced comparable amounts of IgG and IgA production from naive B cells. Moreover, we found that local IgG and IgA levels might be related to IL-17A+ and IL-21+ Tfh cells and IL-4+, IL-21+, and total Tfh cells in nasal tissues, respectively. Therefore the distinct Tfh cell subset enrichment might contribute to the difference in immunoglobulin switching in polypos tissues. Interestingly, the local IgM levels did not correlate with nasal mucosal total Tfh cells or any Tfh cell subset, potentially suggesting a systemic origination rather than local production of IgM in nasal mucosa. Several previous reports have also demonstrated increased IgG and IgA levels in nasal polypos, suggesting that not only IgE but also other immunoglobulins might participate in CRS pathogenesis.25,26

In contrast to nasal polypos tissues, we did not find a marked difference in the frequency and numbers of total CXCR5+CD4+ Tfh cells, the majority of Tfh cell subsets, and B-cell compartments in peripheral blood among the different study groups, which is in line with our previous observation of Tfh1/Tfh2/Tfh17 cell subsets and dendritic cells and further indicates a predominately localized immune response in patients with CRSwNP.27

There are several limitations associated with the present study. First, although we investigated PD1+ and ICOS+ Tfh cells and IFN-γ+, IL-4+, and IL-17+ Tfh cells, respectively, further defining the polypos Tfh cell phenotypes by the combination of surface markers and cytokine expression would deepen our understanding of Tfh cells in inflamed tissues. Second, recently, CXCR5+Bcl-6+ forkhead box P3 (Foxp3)+ follicular regulatory T cells have been identified in GCs with the capacity to suppress GC reactions.28,29 It is critical to add Foxp3 expression to distinguish Tfh cells from Foxp3+ follicular regulatory T cells in future researches.30 Third, the ontogeny and mechanisms of polarization of Tfh cells in the tertiary lymphoid tissues remain to be elucidated.31

These comments notwithstanding, for the first time, our results reflect the existence of expanded Tfh cells in inflamed mucosal tissues from patients with CRSwNP. We suggest that tissue Tfh cells might promote localized B-cell differentiation and proliferation and thus induce local immunoglobulin production in nasal polypos. Mucosal IL-4+ Tfh cells are critical for B-lymphocyte CSR to IgE in nasal polypos. These data not only extend our understanding of the mechanisms of CRSwNP and provide a potential new therapeutic target but also have broad implications for our understanding of the immunopathogenesis of other diseases characterized by the formation of ectopic lymphoid structures and local immunoglobulin production.

Clinical implications: Effective strategies can be designed to target nasal IL-4+ Tfh cells in eosinophilic nasal polypos to suppress local IgE production.

REFERENCES


