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_{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_{FH} cells in eosinophilic nasal polyp tissues compared with noneosinophilic nasal polyp and control normal nasal tissues.

Methods: T_{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_{FH} cells. Sorted nasal T_{FH} cells and CXCR5$^-$ T cells were cultured with autologous naive B cells purified from blood.

Results: Nasal T_{FH} cells expressed inducible costimulator, programmed cell death protein 1, and the transcription factor B-cell lymphoma 6 (Bcl-6) at an intermediate level when compared with *bona fide* T_{FH} cells in tonsils and circulating T_{FH} cells. Although counts of total T_{FH} cells and IL-21$^+$, IFN-γ^+, and IL-17$^+$ T_{FH} cells were increased in both eosinophilic and noneosinophilic nasal polyp tissues compared with those in normal nasal tissues, IL-4$^+$ T_{FH} cell counts were only increased in eosinophilic polyp tissues. IL-4 and IL-21 were involved in polyp T_{FH} cell–induced IgE production from naive B cells, and nasal IL-4$^+$ T_{FH} cell counts correlated highly with local IgE levels *in vivo*. IL-4$^+$Bcl-6$^+$CD4$^+$ T_{FH} cells were identified in ectopic lymphoid structures in eosinophilic nasal polyps. T_{FH} cells also positively correlated with germinal center B cells and plasma cells in nasal tissues.

Conclusion: Nasal IL-4$^+$ T_{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).[1] 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.[2] 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.[3,4] 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 ε germline gene transcript and ε circle transcripts in polyp tissues, strongly suggest local CSR to IgE in patients with eosinophilic CRSwNP.[4-6]

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_H2 cells.[7,8] Recently, it has become clear that a distinct subset of T_H cells beyond the T_H1/T_H2 paradigm, termed T follicular helper (T_{FH}) cells largely on the basis of their localization in B-cell follicles, plays a crucial role in B-cell response induction.[7,8] T_{FH} cells are also distinguishable from other T_H 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_{FH} cells

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Abbreviations used
Bcl-6: Transcription factor B-cell lymphoma 6
Bm: B mature
CRSwNP: Chronic rhinosinusitis with nasal polyps
CSR: Class-switch recombination
FACS: Fluorescence-activated cell sorting
Foxp3: Forkhead box P3
GC: Germinal center
ICOS: Inducible costimulator
NMC: Dispersed nasal mucosal mononuclear cell
PD1: Programmed cell death protein 1
T_{FH}: T follicular helper

in humoral immunity has resulted in many studies designed to understand their roles in, for example, human infections, autoimmune diseases, and vaccination.[9-11] In addition to secondary lymphoid organs, human $CXCR5^+CD4^+$ T_{FH} cells have also been identified in peripheral blood, sharing similar functional properties with *bona fide* GC T_{FH} cells in secondary lymphoid organs and possibly representing a circulating memory compartment of T_{FH} lineage cells.[12-15] Recently, the presence of $CXCR5^+CD4^+$ T_{FH} cells has also been documented in ectopic lymphoid structures in lesional tissues, such as breast cancer and rheumatoid arthritis synovium[16,17]; nevertheless, the phenotypes and functions of these $CXCR5^+CD4^+$ T_{FH} 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 T_{FH} cells in immunoglobulin production, we hypothesized that $CXCR5^+CD4^+$ T_{FH} cells might be present in nasal polyp tissues and involved in local IgE production. In this study we comprehensively evaluated nasal mucosal $CXCR5^+CD4^+$ T_{FH} cell numbers, phenotype, and function in patients with eosinophilic and noneosinophilic CRSwNP. We reported that increased nasal mucosal $IL\text{-}4^+CXCR5^+CD4^+$ T_{FH} cell counts correlate with local IgE production in eosinophilic polyps. Nasal mucosal $CXCR5^+CD4^+$ T_{FH} cells manifest different phenotypic characteristics compared with circulating and *bona fide* T_{FH} 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."[1] CRSwNP was defined as eosinophilic when the percentage of tissue eosinophils exceeded 10% of total infiltrating cells, as reported by our previous study.[2] Subjects undergoing septoplasty because of anatomic variations and without other sinonasal diseases were enrolled as control subjects.[2,4] Patient characteristics are summarized in Table E1 and other additional information is provided in the Methods section in this article's Online Repository at www.jacionline.org.

Histologic study

Hematoxylin and eosin and immunohistochemical staining were conducted, as previously described.[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.

Nasal mucosal mononuclear cell isolation

Sinonasal mucosa were dissociated mechanically with the GentleMACS Dissociator (Miltenyi Biotec Technology & Trading [Shanghai] Co, Shanghai, China).[16] The resulting cell suspension was filtered 2 times through a mesh of 40 μm, and then the dispersed nasal mucosal mononuclear cells (NMCs) were isolated by means of density gradient centrifugation on Lymphoprep (AXIS-SHIELD PoC AS, Oslo, Norway), as previously described.[18]

Flow cytometry

NMCs were obtained, as described above. PBMCs were also isolated by using Lymphoprep (AXIS-SHIELD PoC AS), as previously described.[18] The stained cells were analyzed on a FACSCalibur flow cytometer (Becton Dickinson, San Jose, Calif). Antibodies used are listed in Table E3 and other additional information is provided in the Methods section in this article's Online Repository.

Isolation and purification of naive B cells and T_{FH} cells

Naive $CD19^+IgD^+$ B cells, $CXCR5^-CD4^+$ T cells, and $CXCR5^+CD4^+$ T_{FH} cells were isolated by means of immunomagnetic cell sorting from peripheral blood and nasal polyp tissues, respectively.[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 $CD4^+$ T cells and autologous naive B cells

Sorted T_{FH} cells or $CXCR5^-$ T cells (30×10^3 cells per well) were cocultured with autologous naive B cells (25×10^3 cells per well) for 8 days in U-bottom, 96-well plates, as previously described.[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).[4,20] Total IgG was calculated as the sum of the 4 subclasses, as previously mentioned.[11] Specific IgE to Der p 1 was detected by using the ImmunoCAP system (Phadia, Uppsala, Sweden).[4] 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.[2,4] 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 H test was used to assess significant intergroup variability. The 2-tailed Mann-Whitney U test was used for between-group comparison. The Spearman rank test was used for correlations. Significance was accepted at a 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 α value of $.05/3 = 0.017$ for each comparison.