Ectopic lymphoid tissues support local immunoglobulin production in patients with chronic rhinosinusitis with nasal polyps

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Background: The contribution of ectopic lymphoid tissues (eLTs) to local immunoglobulin hyperproduction in patients with chronic rhinosinusitis with nasal polyps (CRSwNP) is unclear.

Objective: We sought to explore the cellular basis, formation mechanisms, and function of eLTs in patients with CRSwNP.

Methods: We graded lymphoid aggregations in sinonasal mucosa and histologically studied their structures. The expression of lymphorganogenic factors and molecules required for immunoglobulin production was measured by using real-time PCR, and their localization was analyzed by means of immunohistochemistry and immunofluorescence. The phenotype of follicular helper T cells was analyzed by performing flow cytometry. Immunoglobulin levels were quantified by using the Bio-Plex assay or ImmunoCAP system. Nasal tissue explants were challenged *ex vivo* with *Dermatophagoides pteronyssinus* group 1 (Der p 1), and the expression of Iε-Cμ and Iε-Cγ circle transcripts was detected by using seminested PCR.

Results: Increased formation of eLTs with germinal center–like structures was discovered in patients with eosinophilic (20.69%) and noneosinophilic (17.31%) CRSwNP compared with that in patients with chronic rhinosinusitis without nasal polyps (5.66%) and control subjects (3.70%). The presence of eLTs was associated with increased expression of lymphorganogenic and inflammatory chemokines and cytokines, as well as their receptors. The expression of molecules required for immunoglobulin production, generation of follicular helper T cells, and production of IgE in eosinophilic polyps and IgG and IgA in both eosinophilic and noneosinophilic polyps were predominantly upregulated in patients with eLTs. After Der p 1 challenge *ex vivo*, Iε-Cμ transcript was detected only in eosinophilic polyps with eLTs but not in polyps without eLTs and noneosinophilic polyps.

Conclusion: eLTs might support local immunoglobulin production and therefore significantly contribute to the development of CRSwNP. (J Allergy Clin Immunol 2017;■■■:■■■-■■■.)

Key words: *Ectopic lymphoid tissue, immunoglobulin, lymphoid aggregate, lymphorganogenesis*

Chronic rhinosinusitis with nasal polyps (CRSwNP) is characterized by exaggerated inflammation in sinonasal mucosa and polyp formation.[1] Medical treatments usually involve nonspecific suppression of chronic inflammation and are ineffective in many patients, reflecting our limited understanding of the complicated cellular and molecular networks in patients with CRSwNP.[1] An array of evidence has implicated a critical role of local immunoglobulin hyperproduction in the pathogenesis of CRSwNP.[2,3]

IgE antibodies against aeroallergens and *Staphylococcus aureus* enterotoxins were identified in nasal polyps (NPs), which could activate mast cells on allergen exposure and were correlated with local eosinophilia.[4,5] Additionally, autoreactive antibodies, such as anti–double-stranded DNA IgG and anti-BP180 IgG, have been demonstrated recently in NPs and potentially linked with mucosal complement activation.[6-8] Increased IgA levels have also been reported in NPs and might contribute to eosinophil activation.[9] Importantly, higher local IgE and IgG levels were found to associate with poorly controlled disease in patients with CRSwNP, even after surgical intervention.[4,6]

Currently, the mechanisms underlying local immunoglobulin hyperproduction in patients with CRSwNP are poorly understood. Antigen-driven antibody affinity maturation, isotype

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Abbreviations used
AID: Activation-induced cytidine deaminase
BAFF: B cell–activating factor of the TNF family
Bcl: B-cell lymphoma
CD21L: CD21 ligand
CRSsNP: Chronic rhinosinusitis without nasal polyps
CRSwNP: Chronic rhinosinusitis with nasal polyps
DC: Dendritic cell
eLT: Ectopic lymphoid tissue
FDC: Follicular dendritic cell
GC: Germinal center
HEV: High endothelial venule
ICAM-1: Intercellular adhesion molecule 1
ICOS: Inducible costimulator
LT: Lymphotoxin
LTβR: Lymphotoxin β receptor
NP: Nasal polyp
sIgE: Specific IgE
T_{FH}: Follicular helper T
VCAM-1: Vascular cell adhesion molecule 1

switch, and memory formation usually take place within germinal centers (GCs) formed in secondary lymphoid organs, such as spleens or lymph nodes. Nevertheless, increased accumulation and activation of B cells, driven by upregulated expression of B-cell chemokines and activating factors, have been demonstrated in NPs.[9,10] Local IgE levels were upregulated in patients with eosinophilic CRSwNP independent of systemic atopy.[4,11] Moreover, receptor revision and class-switching to IgE could occur in NPs, implicating a potential local antibody response and GC reaction in NPs.[11,12] Indeed, ectopic lymphoid tissues (eLTs) have been found in NPs.[13-16] However, the cellular basis, formation mechanisms, and function of polyp eLTs remain to be defined.

The purpose of this study was to investigate (1) the size and structure of lymphoid aggregates in NPs and their frequency in patients and the relationship between lymphoid aggregates and eLTs characterized by the presence of GC-like structures; (2) the potential mechanisms underlying lymphoid neogenesis in NPs; and (3) the function of eLTs in local immunoglobulin production and its clinical relevance in patients with CRSwNP.

METHODS

Subjects

This study was approved by the Ethics Committee of Tongji Hospital and conducted with written informed consent from each patient. The diagnosis of CRSwNP and chronic rhinosinusitis without nasal polyps (CRSsNP) was made according to the current "European position paper on rhinosinusitis and nasal polyps."[1] CRSwNP was defined as eosinophilic when the percentage of tissue eosinophils exceeded 10% of total infiltrating cells, as reported by our previous study.[17] This cutoff was calculated as twice the SD of the mean of eosinophil percentage in control subjects.[17] Subjects undergoing septoplasty because of anatomic variation and without other sinonasal diseases were enrolled as control subjects.[11,17] Polyp tissues from patients with CRSwNP, diseased sinus mucosa from patients with CRSsNP, and inferior turbinate mucosal tissues from control subjects were obtained during surgery. Demographic characteristics of enrolled subjects are listed in Table E1 in this article's Online Repository at www.jacionline.org, and additional information is provided in the Methods section in this article's Online Repository.

Histology

Tissue sections were stained with hematoxylin and eosin, and lymphocyte aggregation was graded according to a previously described method.[18,19] Each sample was graded according to the highest level of lymphoid aggregation present.[18] Cell aggregates with a radial cell count of between 2 and 5 cells were classified as grade 1, those with 6 to 10 cells were classified as grade 2, and those with more than 10 radial cells were classified as grade 3.[18,19] The number of lymphoid aggregates was counted at a low-power magnification (×100). More information is provided in the Methods section in this article's Online Repository.

Immunohistochemistry and immunofluorescence

Structures of lymphoid aggregates were evaluated by using immunohistochemistry and immunofluorescence, as previously described in prior research.[17,20-22] Antibodies used are listed in Tables E2 and E3 in this article's Online Repository at www.jacionline.org. More information is provided in the Methods section in this article's Online Repository.

Measurement of immunoglobulin, chemokine, and cytokine levels

Immunoglobulin and chemokine levels in tissues and cytokine levels in culture supernatants were detected by using the ImmunoCAP system, Bio-Plex assay, Q-Plex assay, or ELISA, as previously reported.[11,15,23,24] More information is provided in this article's Online Repository, including in Tables E4 to E6 in this article's Online Repository at www.jacionline.org.

RT-PCR

Quantitative RT-PCR was performed with specific primers (see Table E7 in this article's Online Repository at www.jacionline.org), as stated elsewhere.[11,17] The specific circle transcripts Iε-Cμ, Iε-Cγ, Iε-Cγ1, Iε-Cγ3, and Iε-Cγ4 were investigated by using a seminested PCR with appropriate primers (see Table E8 in this article's Online Repository at www.jacionline.org), as previously described.[11,25] More information is provided in the Methods section in this article's Online Repository.

Ex vivo allergen challenge

Sinonasal mucosal tissues obtained from 5 control subjects without local specific IgE (sIgE) against Der p 1 and not having eLTs, 9 patients with eosinophilic CRSwNP with local sIgE against Der p 1 (5 of them having eLTs in NPs), and 8 patients with noneosinophilic CRSwNP without local sIgE against Der p 1 (4 of them having eLTs in NPs) were subject to *ex vivo* air-liquid interface culture.[25-27] Tissue explants were stimulated with rDer p 1 (20 μg/mL, endotoxin ≤0.03 EU/μg; Indoor Biotechnologies, Charlottesville, Va) for 24 hours, as mentioned previously.[25-27] After stimulation, tissue explants and culture supernatants were harvested for further analysis. More information is provided in the Methods section in this article's Online Repository.

Flow cytometry

Flow cytometric analysis of nasal mucosal mononuclear cells was performed, as previously described.[15,20] More information is provided in Table E9 in this article's Online Repository at www.jacionline.org.

Statistics

Statistical analysis was performed with SPSS 18.0 software (SPSS, Chicago, Ill). For continuous variables, results are presented in dot plots, unless stated specifically. Symbols represent individual samples, horizontal bars represent medians, and error bars show interquartile ranges. When comparisons were made between groups, the Kruskal-Wallis H test was used to assess significant intergroup variability. The Mann-Whitney U 2-tailed test was used for between-group comparison. Fisher exact or χ^2 tests were applied to compare