Local expression of interleukin-17a is correlated with nasal eosinophilia and clinical severity in allergic rhinitis

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ABSTRACT

Interleukin (IL)-17A is a major cytokine produced by Th17 cells, which are associated with chronic inflammations. The local expression of IL-17A in allergic rhinitis (AR) remains to be characterized. We sought to determine the role of IL-17A expression in human inferior turbinate mucosa in the pathophysiology of AR. Inferior turbinate mucosa was sampled from medical treatment–resistant, surgery-required patients with perennial AR (PAR, n = 21), nonallergic rhinitis with eosinophilia syndrome (NARES, n = 7), and nonallergic hypertrophic rhinitis (HR, n = 13). IL-17A expression was determined with immunohistochemical staining. The mean number of IL-17A$^+$ cells and eosinophils per field were counted. Total serum immunoglobulin E (IgE) levels, blood eosinophil count, and forced expiratory volume in 1 second (FEV$_1$)/forced vital capacity (FVC) ratio were also examined in each patient. IL-17A was primarily expressed in infiltrating inflammatory cells. The number of IL-17A$^+$ cells in nasal mucosa was significantly higher in the PAR group compared with HR (p = 0.002) and NARES (p = 0.021) groups. There was a significant and positive correlation between the number of IL-17A$^+$ cells and total nasal symptom score (rho = 0.403; p = 0.011), especially sneezing score (rho = 0.471; p = 0.003). The number of IL-17A$^+$ cells was significantly and positively correlated with the degree of eosinophil infiltration (rho = 0.623; p < 0.001), but not with total serum IgE levels (rho = 0.284; p = 0.098), blood eosinophil counts (rho = 0.302; p = 0.056), or FEV$_1$/FVC ratio (rho = 0.092; p = 0.569). The present study provides evidence that IL-17A expression in the nasal mucosa is associated with the pathophysiology of AR, including disease severity and nasal eosinophilia.

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Allergic rhinitis (AR) is the inflammation of nasal mucosa, and allergen-specific CD4$^+$ Th2 cells, which produce interleukin (IL)-4, IL-5, IL-13, and IL-31, are believed to play a central role in its pathogenesis.[1,2] Other CD4$^+$ T-cell subsets, including Th1, Tr1, and Treg cells, can regulate Th2 responses and inflammation of AR.[3–5] Recently, Th17 cells were characterized as a distinct lineage of CD4$^+$ T cells and were found to be associated with autoimmune diseases and involved in the protection against microbial infections and chronic inflammation.[6,7]

IL-17A is a proinflammatory cytokine synthesized by Th17 cells.[6] IL-17A acts on a broad range of respiratory cells to induce the expression of cytokines, chemokines, matrix metalloproteinase proteins, and mucus proteins.[8,9] For example, we have recently reported that IL-17A expression is associated with eosinophilic inflammation in chronic rhinosinusitis (CRS) both *in vivo* and *ex vivo*.[10]

Research on the role of IL-17A in the pathogenesis of AR has accumulated.[11–19] Ciprandi *et al.* showed that serum concentrations of IL-17A are significantly elevated in patients with AR compared with healthy controls, and a significant positive relationship between serum IL-17A levels and symptom severity was observed.[11,12] They also found that peripheral blood mononuclear cells from AR patients have higher frequencies of IL-17A producing T cells and CD161$^+$ circulating T cells compared with those from normal subjects.[13,16] Neiminen *et al.* indicated that specific allergen-induced IL-17A mRNA expression in peripheral blood mononuclear cells of pediatric patients with AR was significantly and positively correlated with the symptom–medication score.[14] Additionally, Xu *et al.* showed that IL-17A levels in nasal lavages of patients with AR were significantly higher compared with those of controls. They further indicated that IL-17A enhanced CCL-20 and IL-8 expression in human nasal epithelial cells.[15] More recently, Baumann *et al.* found a significant increase of IL-17A in the nasal lavages of patients with seasonal AR after nasal allergen challenge.[20] On the other hand,

Table 1 Subject characteristics

	HR	NARES	PAR
No. of patients	13	7	21
Sex (male/female)	11/2	6/1	16/5
Age (yr)	37 (24–57)	44 (21–58)	31 (20–57)
CAP titer to *Dermatophagoides farinae* (UA/mL)	0 (0–0)	0 (0–0)	42.2 (0.55 to >100)
Total IgE (IU/mL)	77 (5–182)	149 (36–280)	1139 (24–9831)
Blood eosinophil (μL^{-1})	171 (68–610)	156 (34–305)	409 (37–1754)
FEV_1/FVC ratio	82 (70–100)	82 (72–92)	86 (67–100)
Patients with asthma	0	0	3

HR = hypertrophic rhinitis; NARES = nonallergic rhinitis with eosinophilic syndrome; PAR = perennial allergic rhinitis; IgE = immunoglobulin E; FEV_1 = forced expiratory volume 1 s; FVC = forced vital capacity.

Groger *et al.* showed that elevated levels of IL-17A in nasal secretions were found in patients with nonallergic rhinitis with eosinophilia syndrome (NARES) compared with healthy controls as well as AR.[17] Mouse models have shown that IL-17A contributes to the development and regulation of AR.[18,19,21] However, local IL-17A expression in AR remains to be characterized.

In the present study, we sought to determine the expression of IL-17A in human inferior turbinate mucosa and compared the expression between patients with AR and nonallergic rhinitis. Furthermore, we analyzed the correlations between IL-17A expression in nasal mucosa and various pathophysiological parameters. We believe that the results presented here may provide insight into the role of IL-17A and Th17 in the pathophysiology of AR.

MATERIALS AND METHODS

Patients

Twenty-one Japanese patients with perennial AR (PAR), 7 patients with NARES, and 13 patients with nonallergic hypertrophic rhinitis (HR) were enrolled in the study. All of them presented with persistent nasal obstruction, were resistant to medical treatment, and underwent endoscopic nasal surgery (inferior turbinectomy with or without septoplasty). PAR and NARES were defined based on the Practical Guideline for Management of Allergic Rhinitis in Japan.[22] Patients having CRS were excluded. All of the PAR patients were sensitized with *Dermatophagoides farinae*, as confirmed by the presence of specific immunoglobulin E (IgE) antibodies (range, 0.55 to >100 UA/mL; mean, 42.22 ± 40.54 UA/mL), which were detected *via* an ImmunoCAP kit (Phadia AB, Uppsala, Sweden). Conversely, HR patients did not show nasal eosinophilia or sensitization to airborne allergens related to the symptoms. Three patients with PAR were asthmatic, and no patients had aspirin sensitivity. None of the participants received systemic steroid treatment for a period of at least 8 weeks before surgery, and none received pharmacotherapy for rhinitis, such as intranasal steroids, for a period of at least 3 weeks before surgery. Patients treated with allergen-specific immunotherapy were excluded. Before surgery, we examined the total serum IgE levels, blood eosinophil count, and forced expiratory volume in 1 second/forced vital capacity ratio in each patient. The severity of nasal symptoms was graded according to the criteria outlined by Okuda *et al.*, in which three nasal symptoms (*i.e.*, sneezing, rhinorrhea, and nasal congestion) were rated on a 4-point scale from 0 to 3 (0 = no symptoms, 1 = minimal, well-tolerated symptoms, 2 = bothersome but tolerated symptoms, 3 = severe and hard to tolerate symptoms).[23] The clinical characteristics of the patients are presented in Table 1. All patients provided informed consent before their participation, and the study was approved by the Human Research Committee of the Okayama University Graduate School of Medicine and Dentistry.

Immunohistochemistry

During surgery, the mucosa of the inferior turbinate was sampled from all of the patients. We performed immunohistochemical staining for IL-17A, according to a previously described protocol.[22] Briefly, 4-μm sections were collected from paraffin-embedded tissue blocks, deparaffinized, and rehydrated. The sections were incubated with trypsin for antigen retrieval and primary antibody, including 1:50 diluted rabbit anti-human IL-17A polyclonal antibody (H-132; Santa Cruz Biotechnology, Santa Cruz, CA) or control serum (Universal Negative Control; Dako Japan, Tokyo, Japan), at 4°C overnight. A Histofine MAX-PO(R) (Nichirei Bioscience, Tokyo, Japan) with diaminobenzidine substrate was used according to the manufacturer's instructions. The sections were then nuclear stained with hematoxylin and examined under a light microscope.
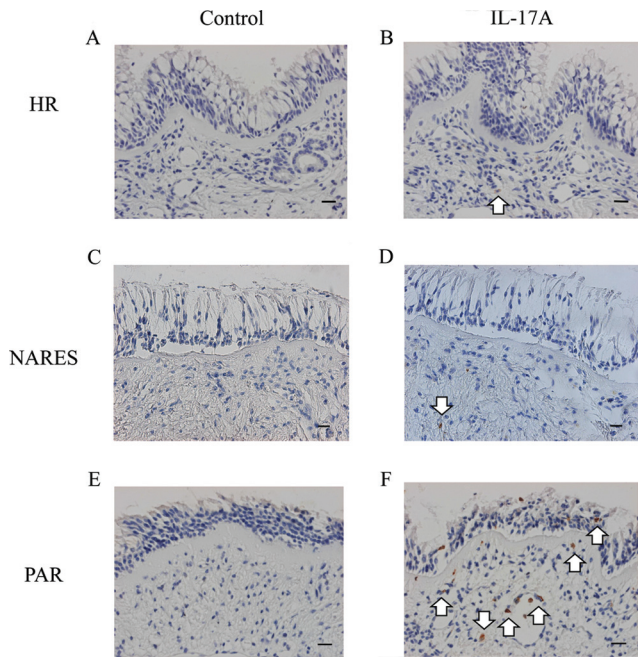
Figure 1. *Immunohistochemical staining of interleukin (IL)-17A in nasal mucosa from a patient with (A and B) nonallergic hypertrophic rhinitis (HR), (C and D) nonallergic rhinitis with eosinophilia syndrome (NARES), and (E and F) perennial allergic rhinitis (PAR). Sections were reacted with rabbit polyclonal antibody against (B, D, and F) IL-17A (A, C, and E) or control after which they were stained using a Histofine MAX-PO (Nichirei Bioscience, Tokyo, Japan) with a diaminobenzidine substrate. Arrows indicate IL-17+ cells (scale bar = 20 μm).*

Positive-stained cells were counted in five fields at high power (10 × 40), where the highest cellular infiltration was observed. The mean number of positive cells was then determined. Additionally, sections were stained with hematoxylin and eosin, and the number of eosinophils that had infiltrated into the nasal mucosa was counted in the same manner.

Statistical Analysis

Values are presented as median values. A nonparametric Mann-Whitney *U* test was used for comparing data between groups. A correlation analysis was performed using a nonparametric Spearman's correlation coefficient by rank. A value of $p < 0.05$ was considered statistically significant. Statistical analyses were performed using StatView software (Version 4.5; Abacus Concepts, Berkeley, CA).

RESULTS

Local Expression of IL-17A in Nasal Mucosa

We immunohistochemically examined the expression and distribution of IL-17A in the nasal mucosa of inferior turbinate. IL-17A protein was primarily expressed in infiltrating inflammatory cells, but not epithe-
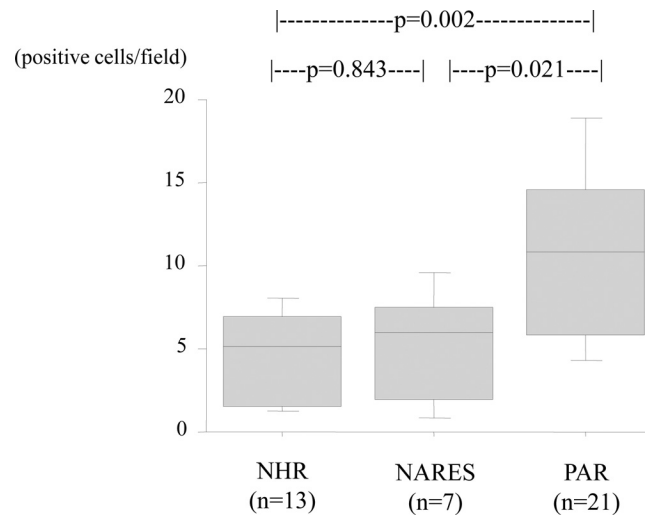

Figure 2. *Comparison of numbers of interleukin (IL)-17A$^+$ cells in the nasal mucosa among patients with nonallergic hypertrophic rhinitis (HR), nonallergic rhinitis with eosinophilia syndrome (NARES), and perennial allergic rhinitis (PAR). The rectangle includes the range from the 25th to the 75th percentiles, the horizontal line indicates the median, and the vertical line indicates the range from the 10th to 90th percentiles. The p values were determined by the Mann-Whitney U test.*

lial cells, vascular endothelial cells, glands, or fibroblasts. The expression levels of IL-17A were determined in every group, with a greater expression observed in the PAR group versus the HR and NARES groups (Fig. 1). The number of IL-17A$^+$ cells in the nasal mucosa was significantly higher in the PAR group compared with HR ($p = 0.002$) and NARES ($p = 0.021$) groups. Conversely, the number was similar between the HR and NARES group ($p = 0.843$; Fig. 2).

Pathophysiological Significance of IL-17A Expression in Nasal Mucosa

A significant and positive correlation was seen between the number of IL-17A$^+$ cells in the nasal mucosa and total nasal symptom score, which was determined from the sum of sneezing, rhinorrhea, and congestion scores ($\rho = 0.403$; $p = 0.011$; Fig. 3 *A*). In detail, the sneezing score *per se* was significantly and positively correlated with the number of IL-17A$^+$ cells ($\rho = 0.471$; $p = 0.003$; Fig. 3 *B*), whereas the rhinorrhea ($\rho = 0.291$; $p = 0.066$; Fig. 3 *C*) and congestion ($\rho = 0.206$; $p = 0.192$; Fig. 3 *D*) were not.

The number of IL-17A$^+$ cells did not correlate with total serum IgE levels ($\rho = 0.284$; $p = 0.098$; Fig. 3 *E*), blood eosinophil counts ($\rho = 0.302$, p = .056, Fig. 3 *F*), or forced expiratory volume in 1 second/forced vital capacity ratio ($\rho = 0.092$; $p = 0.569$; Fig. 3 *G*). However, the degree of eosinophil infiltration into the nasal mucosa was significantly and positively
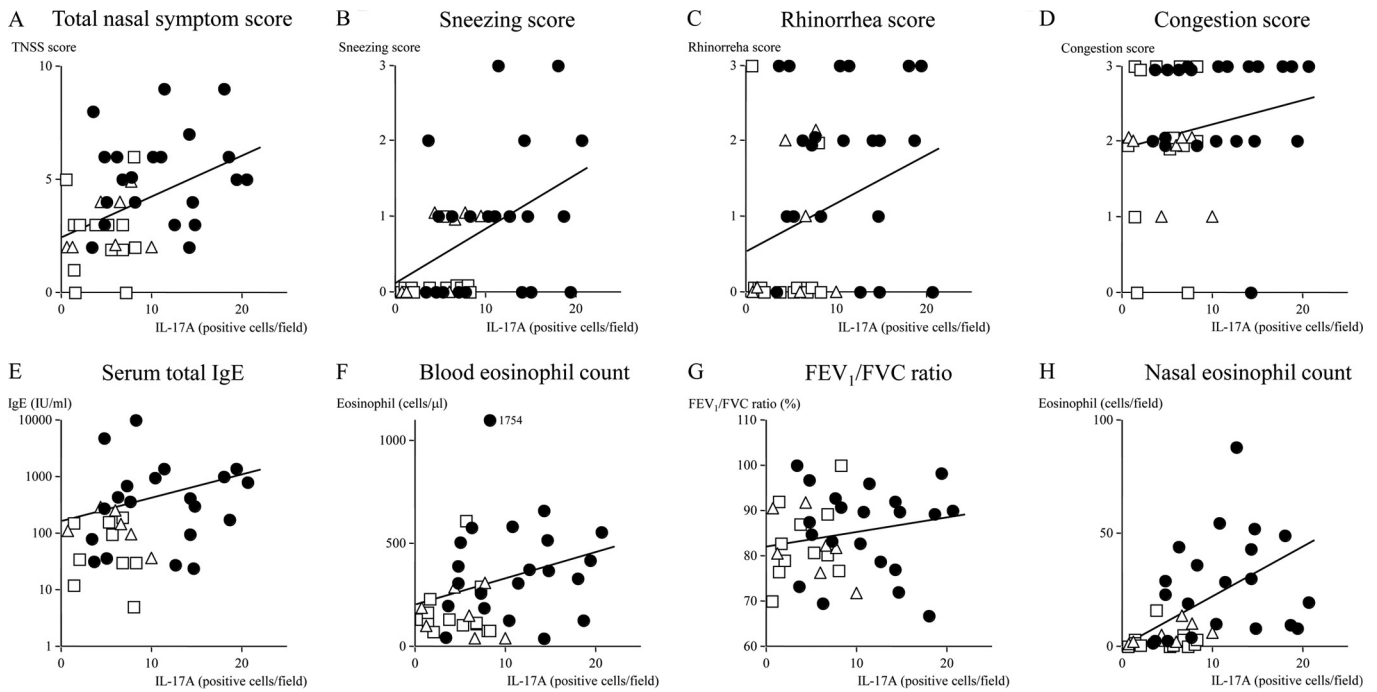
Figure 3. *Relationship between numbers of interleukin (IL)-17A$^+$ cells in nasal mucosa and pathophysiological characterizations including (A) total nasal symptom score (TNSS), (B) sneezing score, (C) rhinorrhea score, (D) congestion score, (E) serum total IgE levels, (F) blood eosinophil count, (G) forced expiratory volume in 1 second/forced vital capacity (FEV$_1$/FVC) ratio, and (H) number of eosinophils in nasal mucosa. Nasal mucosa were sampled from patients with non-allergic hypertrophic rhinitis (HR; □), nonallergic rhinitis with eosinophilia syndrome (△), and perennial allergic rhinitis (PAR; ●).*

correlated with the number of IL-17A$^+$ cells (ρ = 0.623; $p < 0.001$; Fig. 3 *H*).

DISCUSSION

In the present study, we characterized the expression of IL-17A in the pathogenesis of AR. Our findings suggest that the expression of IL-17A in the nasal mucosa of the inferior turbinate is associated with not only local eosinophilic inflammation, but also severity of nasal symptoms.

Although we and others have previously shown the expression of IL-17A protein in nasal polyps, this was first studied in the English language to clarify the expression in human inferior turbinate mucosa.[10,25,26] We found three Chinese articles (with English abstract), which reported on IL-17, although they had different results and it was unclear whether they assessed IL-17A or other members of the IL-17 cytokine family.[27–29] Ba *et al.* showed that the number of IL-17$^+$ cells in tissues of AR patients was higher than that of controls ($p < 0.05$). They also showed that the eosinophilic cell count correlated with the number of IL-17$^+$ cells ($r = 0.446$; $p < 0.05$).[27] Interestingly, in a different report, the same authors described that the expression of IL-17 was only apparent in the nasal mucosa of patients with AR.[28] Liu *et al.* reported that there were no significant differences between AR and nonallergic rhinitis patients in the protein expression of IL-17 in

inferior turbinate tissues.[29] Local IL-17A expression in the sinonasal mucosa is known to have ethnic and/or regional differences.[30] A recent study by Katotomichelakis *et al.* strengthens the importance of studies in the Asian population because there is a difference in cytokine profile in European populations that changes over time.[31] If IL-17 actually refers to IL-17A in these articles, then our findings corroborate those of Ba *et al.* and suggest that the local expression of IL-17A is elevated in AR patients, especially in Asia.

The expression of IL-17A in the inferior turbinate mucosa was found positively correlated with the degree of local eosinophilia. We previously reported that the number of IL-17A$^+$ cells in sinonasal tissues was positively correlated with the degree of local eosinophilia in CRS.[10] These results suggest that the local expression of IL-17A is associated with eosinophilic inflammation in both AR and CRS. In this study, we also showed that IL-17A directly induces the production of IL-6 and granulocyte macrophage colony-stimulating factor *via* dispersed nasal polyp cells, both of which are known to promote eosinophilic inflammation.[32,33] Although it remains unclear as to how IL-17A drives eosinophilic inflammation in AR, nasal eosinophilia in AR may be induced *via* a cytokine orchestration, in which IL-17A is involved. Bachert *et al.* showed the importance of IgE levels in tissue for the treatment strategy in CRS.[34] An important finding of this

study is that IL-17A$^+$ cells were significantly and positively correlated with the degree of eosinophil infiltration, but not with total serum IgE levels and blood eosinophil counts. This emphasizes the need of counting IgE levels and eosinophils in tissue rather than blood.

The number of IL-17A$^+$ cells in the nasal mucosa was significantly correlated with the total nasal symptom score, suggesting that IL-17A is closely associated with the disease severity of AR. This result was consistent with previous reports showing that serum IL-17A levels and allergen-induced IL-17A mRNA expression correlate with symptom severity, as assessed *via* a visual analog scale and symptom medication score, respectively.[12,14] When we analyzed the individual symptoms separately, only the sneezing score correlated with the number of IL-17A$^+$ cells. The sneezing reflex, which follows an allergen challenge, is primarily a respiratory reflex induced by the interaction between histamine and the H$_1$-receptor at the sensory nerve terminals.[35] Eosinophil infiltration into the nasal mucosa induces a minimal persistent inflammation and the priming effect, both of which can amplify nasal hyperreactivity.[36] Thus, the expression of IL-17A may induce sneezing *via* an indirect enhancement of eosinophilic inflammation. On the other hand, the congestion score did not correlate with IL-17A expression. From an ethical view, it is hard to sample the inferior turbinate mucosa from patients complaining of slight congestion. One of the reasons why the congestion score did not correlate with the IL-17A expression may be because only subjects with medical treatment–resistant, surgery-required swollen inferior turbinates were included in the present study.

In nasal polyps, we performed double immunofluorescence staining and found that CD68$^+$ cells, CD4$^+$ cells, and EG2$^+$ cells expressed IL-17A.[10] Although we could not find such an investigation in the inferior turbinate, both mononuclear and polynuclear cells expressed IL-17A in the immunohistochemistry, suggesting that infiltrating inflammatory cells such as lymphocytes, plasma cells, macrophages, mast cells, and eosinophils may produce IL-17A in AR.

In conclusion, the present study provides evidence that local IL-17A expression is associated with the pathophysiology of AR, including disease severity and nasal eosinophilia. These observations may provide a basis for future therapeutic approaches targeting IL-17A in the management of severe eosinophilic airway diseases, including AR, CRS with nasal polyps, and bronchial asthma.

ACKNOWLEDGMENTS

REFERENCES

1. Durham SR, Ying S, Varney VA, et al. Cytokine messenger RNA expression for IL-3, IL-4, IL-5, and granulocyte/macrophage colony-stimulating factor in the nasal mucosa after local allergen provocation: Relationship to tissue eosinophilia. J Immunol 148:2390–2394, 1992.
2. Okano M, Fujiwara T, Higaki T, et al. Characterization of pollen antigen-induced IL-31 production by PBMCs in allergic rhinitis. J Allergy Clin Immunol 127:277–279, 279.e1–e11, 2011.
3. Yamanaka K, Yuta A, Kakeda M, et al. Induction of IL-10-producing regulatory T cells with TCR diversity by epitope-specific immunotherapy in pollinosis. J Allergy Clin Immunol 124:842–845.e7, 2009.
4. Prigione I, Morandi F, Tosca MA, et al. Interferon-gamma and IL-10 may protect from allergic polysensitization in children: Preliminary evidence. Allergy 65:740–742, 2010.
5. Skrindo I, Scheel C, Johansen FE, and Jahnsen FL. Experimentally induced accumulation of Foxp3$^+$ T cells in upper airway allergy. Clin Exp Allergy 41:954–962, 2011.
6. Louten J, Boniface K, de Waal Malefyt R. Development and function of TH17 cells in health and disease. J Allergy Clin Immunol 123:1004–1011, 2009.
7. Cosmi L, Liotta F, Maggi E, et al. Th17 cell: New players in asthma pathogenesis. Allergy 66:989–998, 2011.
8. Wang YH, AND Liu YJ. The IL-17 cytokine family and their role in allergic inflammation. Curr Opin Immunol 20:697–702, 2008.
9. Nembrini C, Marsland BJ, and Kopf M. IL-17-producing T cells in lung immunity and inflammation. J Allergy Clin Immunol 123:986–994, 2009.
10. Makihara S, Okano M, Fujiwara T, et al. Regulation and characterization of IL-17A expression in patients with chronic rhinosinusitis and its relationship with eosinophilic inflammation. J Allergy Clin Immunol 126:397–400, 400.e1–11, 2010.
11. Ciprandi G, Fenoglio D, De Amici M, et al. Serum IL-17 levels in patients with allergic rhinitis. J Allergy Clin Immunol 122:650–651.e2, 2008.
12. Ciprandi G, De Amici M, Murdaca G, et al. Serum interleukin-17 levels are related to clinical severity in allergic rhinitis. Allergy 64:1375–1378, 2009.
13. Ciprandi G, Filaci G, Battaglia F, and Fenoglio D. Peripheral Th-17 cell in allergic rhinitis: New evidence. Int Immunopharmacol 10:226–229, 2009.
14. Nieminen K, Valovirta E, and Savolainen J. Clinical outcome and IL-17, IL-23, IL-27 and FOXP3 expression in peripheral blood mononuclear cells of pollen-allergic children during sublingual immunotherapy. Pediatr Allergy Immunol 21:e174–e184, 2010.
15. Xu G, Zhang L, Wang DY, et al. Opposing roles of IL-17A and IL-25 in the regulation of TSLP production in human nasal epithelial cells. Allergy 65:581–589, 2010.
16. Poggi A, Canevali P, Contatore M, and Ciprandi G. Higher frequencies of CD161+ circulating T lymphocytes in allergic rhinitis compared to healthy donors. Int Arch Allergy Immunol 158:151–156, 2012.
17. Gröger M, Klemens C, Wendt S, et al. Mediators and cytokines in persistent allergic rhinitis and nonallergic rhinitis with eosinophilia syndrome. Int Arch Allergy Immunol 159:171–178, 2012.
18. Quan SH, Zhang YL, Han DH, et al. Contribution of interleukin 17A to the development and regulation of allergic inflammation in a murine allergic rhinitis model. Ann Allergy Asthma Immunol 108:342–350, 2012.
19. Moon IJ, Hong SL, Kim DY, et al. Blocking interleukin-17 attenuates enhanced inflammation by staphylococcal enterotoxin B in murine allergic rhinitis model. Acta Otolaryngol 132(suppl 1):S6–S12, 2012.

20. Baumann R, Rabaszowski M, Stenin I, et al. Comparison of the nasal release of IL-4, IL-10, IL-17, CCL13/MCP-4, and CCL26/eotaxin-3 in allergic rhinitis during season and after allergen challenge. Am J Rhinol Allergy 27:266–272, 2013.

21. Wang M, Zhang W, Shang J, et al. Immunomodulatory effects of IL-23 and IL-17 in a mouse model of allergic rhinitis. Clin Exp Allergy 43:956–966, 2013.

22. Okubo K, Kurono Y, Fujieda S, et al. Japanese guideline for allergic rhinitis. Allergol Int 60:171–189, 2011.

23. Okuda M, Okamoto M, Nomura Y, and Saito Y. Clinical study on beclomethasone dipropionate powder preparation (TL-102) in perennial nasal allergy. Rhinology 24:113–123, 1986.

24. Okano M, Fujiwara T, Yamamoto M, et al. Role of prostaglandin D_2 and E_2 terminal synthases in chronic rhinosinusitis. Clin Exp Allergy 36:1028–1038, 2006.

25. Van Bruaene N, Pérez-Novo CA, Basinski TM, et al. T-cell regulation in sinus diseases. J Allergy Clin Immunol 121:1435–1441, 2008.

26. Jiang XD, Li GY, Li L, et al. The characterization of IL-17A expression in patients with chronic rhinosinusitis with nasal polyps. Am J Rhinol Allergy 25:e171–e175, 2011.

27. Ba L, Du J, Liu Y, et al. The expression and significance of interleukin-17 and the infiltrating eosinophils in nasal polyps and nasal mucous of allergic rhinitis [in Chinese]. Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 24:53–54, 2010.

28. Du JT, Ba L, Shang TT, et al. The expression of IL-17 in blood and nasal tissue of patients with allergic rhinitis and nasal polyps. Sichuan Da Xue Xue Bao Yi Xue Ban 41:235–238, 2010.

29. Liu Z, Lu X, Wang H, et al. The expression of transforming growth factor beta1, interleukin-6, 11 and 17 in nasal mucosa of allergic rhinitis patients. Lin Chuang Er Bi Yan Hou Ke Za Zhi 20:625–627, 2006.

30. Zhang N, Van Zele T, Perez-Novo C, et al. Different types of T-effector cells orchestrate mucosal inflammation in chronic sinus disease. J Allergy Clin Immunol 122:961–968, 2008.

31. Katotomichelakis M, Tantilipikorn P, Holtappels G, et al. Inflammatory patterns in upper airway disease in the same geographical area may change over time. Am J Rhinol Allergy 27:354–360, 2013.

32. Su YC, Rolph MS, Hansbro NG, et al. Granulocyte-macrophage colony-stimulating factor is required for bronchial eosinophilia in a murine model of allergic airway inflammation. J Immunol 180:2600–2607, 2008.

33. Doganci A, Eigenbrod T, Krug N, et al. The IL-6R alpha chain controls lung CD4+CD25+ Treg development and function during allergic airway inflammation in vivo. J Clin Invest 115:313–325, 2005.

34. Bachert C, Gevaert P, Holtappels G, et al. Total and specific IgE in nasal polyps is related to local eosinophilic inflammation. J Allergy Clin Immunol 107:607–614, 2001.

35. Okano M. Mechanisms and clinical implications of glucocorticosteroids in the treatment of allergic rhinitis. Clin Exp Immunol 158:164–173, 2009.

36. Canonica GW, and Compalati E. Minimal persistent inflammation in allergic rhinitis: implications for current treatment strategies. Clin Exp Immunol 158:260–271, 2009. □