IL-25 as a novel therapeutic target in nasal polyps of patients with chronic rhinosinusitis

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Background: Chronic rhinosinusitis (CRS) with nasal polyps (NPs) in Western populations is associated with T_H2 cytokine polarization. IL-25, an IL-17 family cytokine, was recently reported to induce T_H2-type immune responses and to contribute to several allergic diseases, such as atopic dermatitis and asthma. However, the role of IL-25 in Asian patients with nasal polyposis remains unclear.

Objective: We sought to determine the role of IL-25 in Asian patients with nasal polyposis and CRS.

Methods: We investigated IL-25 expression and its cellular origins in NPs of human subjects using immunohistochemistry (IHC), quantitative RT-PCR, and ELISA of NP tissues. Correlations between IL-25 expression and expression of other inflammatory markers in NP tissues were also explored. Anti–IL-25 neutralizing antibody was administered in an ovalbumin- and staphylococcal enterotoxin B–induced murine NP model to confirm the function of IL-25 during nasal polypogenesis.

Results: IL-25 expression was upregulated in NP mucosa from patients with CRS with NPs compared with uncinate process tissue from control subjects and those with CRS without NPs. Overexpression of epithelial IL-25 was confirmed by using IHC, and double IHC staining showed that tryptase-positive cells were one of the main sources of IL-25 among immune cells. Furthermore, IL-17 receptor B levels were also increased in immune cells of patients with NPs compared with those in control subjects. In NPs IL-25 mRNA expression positively correlated with the expression of several inflammatory markers, including T-box transcription factor, RAR-related orphan receptor C, GATA3, eosinophil cationic protein, TGF-β1, and TGF-β2. IL-25 was more abundant in the murine NP model compared with control mice, and similar correlations between IL-25 and inflammatory markers were observed in murine models. Anti–IL-25 treatment reduced the number of polyps, mucosal edema thickness, collagen deposition, and infiltration of inflammatory cells, such as eosinophils and neutrophils. This treatment also inhibited expression of local inflammatory cytokines, such as IL-4 and IFN-γ. Furthermore, expression of CCL11, CXCL2, intercellular adhesion molecule 1, and vascular cell adhesion molecule 1 in the nasal mucosa was suppressed in the anti–IL-25–treated group.

Conclusion: Our results suggest that IL-25 secreted from the sinonasal epithelia and infiltrating mast cells plays a crucial role in the pathogenesis of CRS with NPs in Asian patients. In addition, our results suggest the novel possibility of treating nasal polyposis with anti–IL-25 therapy. (J Allergy Clin Immunol 2015;135:1476-85.)

Key words: *Nasal polyp, IL-25, sinusitis, allergy, animal models*

Chronic rhinosinusitis (CRS) is a common upper airway disease that affects 5% to 16% of the population worldwide.[1-3] CRS is characterized by chronic inflammation of the sinonasal mucosa that persists for at least 12 weeks despite medical treatment.[2] Nasal polyps (NPs) frequently accompany CRS, and their occurrence indicates a more serious illness with recurrent clinical phenotypes.[4,5] Chronic rhinosinusitis with nasal polyps (CRSwNP) in Western populations is associated with T_H2 cytokine polarization and prominent eosinophilic infiltration.[6] Thus upstream mechanisms that incite the T_H2 response are crucial for understanding the pathogenesis of CRSwNP[7]; however, these mechanisms are not fully understood.

IL-25 (also known as IL-17E) is a member of the IL-17 cytokine family and has been reported to play a variety of roles in different inflammatory murine models, such as asthma, atopic dermatitis, and pulmonary fibrosis. Intraperitoneal or intranasal administration of IL-25 protein resulted in the production of eosinophils or T_H2 cytokines, such as IL-4, IL-5, IL-13, and eotaxin, in bronchoalveolar lavage fluid and lung tissue.[8,9] Conversely, blocking IL-25 decreases T_H2 cytokine production in an animal model of asthma.[10-12] In addition, IL-25 could function in patients with allergic dermatitis by inducing the T_H2

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Supported by a clinical research grant-in-aid from the Seoul Metropolitan Government Seoul National University (SMG-SNU) Boramae Medical Center (03-2013-7), by SK Telecom Research Fund (06-2012-109), and by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (0411-20130037).

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication June 12, 2014; revised December 30, 2014; accepted for publication January 12, 2015.

Available online February 25, 2015.

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0091-6749/$36.00

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http://dx.doi.org/10.1016/j.jaci.2015.01.003

Abbreviations used
CRS: Chronic rhinosinusitis
CRSsNP: Chronic rhinosinusitis without nasal polyps
CRSwNP: Chronic rhinosinusitis with nasal polyps
CT: Computed tomography
ECP: Eosinophil cationic protein
hpf: High-power field
ICAM: Intercellular adhesion molecule
IHC: Immunohistochemistry
IL-17R: IL-17 receptor
NP: Nasal polyp
OVA: Ovalbumin
RORC: RAR-related orphan receptor C
SEB: Staphylococcal enterotoxin B
T-bet: T-box transcription factor
UP: Uncinate process
VCAM: Vascular cell adhesion molecule

response, as well as by inhibiting filaggrin synthesis, consequently affecting skin barrier function.[13,14] Apart from allergic diseases, IL-25 also plays an important role in different inflammatory conditions. One recent study showed that expression of IL-25 was increased and correlated with levels of periostin, an extracellular matrix protein, in patients with idiopathic pulmonary fibrosis.[15] Moreover, this study demonstrated that IL-25 can drive fibrosis, which was confirmed by a decrease in collagen deposition in IL-25–deficient animal models.[15] Collectively, these data indicate that IL-25 is a potent cytokine that acts in diverse inflammatory conditions.

Recently, IL-25 and IL-33, which are both produced by sinonasal epithelial cells, were reported to have critical roles in promoting T_H2-mediated inflammation.[16] Induction of IL-33, a member of the IL-1 cytokine family, has been observed in epithelial cells from patients with CRSwNP. IL-33 induction also stimulates IL-13 production in $ST2^+$ innate lymphoid cells from NPs.[17] IL-25 enhances thymic stromal lymphopoietin–induced T_H2 cell expansion and function.[18] Although one study reported that increased IL-25 levels correlated with poorer computed tomographic (CT) scores and increased serum eosinophil numbers in sinus mucosal tissues in patients with CRS, thus suggesting a relationship between IL-25 and T_H2-dominant diseases,[19] the specific role of IL-25 in Asian patients with CRSwNP has not been thoroughly explored. In this study we investigated the expression and cellular origin of IL-25, as well as correlations between IL-25 and inflammatory surrogates in sinonasal tissues from patients with CRSwNP. We also evaluated the effects of anti–IL-25 therapy on nasal polyp formation in an NP-induced murine model. Some results of this study have been previously reported in the form of an abstract.[20]

METHODS

Patients and tissue samples

Sinonasal and polyp tissues were obtained from routine functional endoscopic sinus surgery in patients with CRS. CRS diagnoses were based on personal medical history, physical examination, nasal endoscopy, and CT findings of the sinuses according to the "EPOS 2012: European position paper on rhinosinusitis and nasal polyps 2012" guidelines.[21] Patients were excluded if they were (1) younger than 18 years old; (2) asthmatic or aspirin sensitive; (3) previously treated with antibiotics, systemic or topical corticosteroids, or other immune-modulating drugs up to 4 weeks before surgery; and (4) afflicted with conditions, such as unilateral rhinosinusitis, antrochoanal polyps, allergic fungal rhinosinusitis, cystic fibrosis, or immotile ciliary disease. Control tissues were obtained from patients without any sinonasal disease during other rhinologic surgeries, such as skull base, lacrimal duct, or orbital decompression surgery. We also obtained uncinate process (UP) tissue from control subjects and patients with CRS, including those with chronic rhinosinusitis without nasal polyps (CRSsNP) and those with CRSwNP. We also evaluated NPs in patients with CRSwNP. Each sample obtained was divided into 3 parts: one part was fixed in 10% formaldehyde and embedded in paraffin for histologic analyses, another part was immediately frozen and stored at −80°C for subsequent isolation of mRNA and proteins, and the third part was submersed in 1 mL of PBS supplemented with 0.05% Tween 20 (Sigma-Aldrich, St Louis, Mo) and 1% PIC (Sigma-Aldrich) per 0.1 g of tissue. This tissue was homogenized with a mechanical homogenizer at 1000 rpm for 5 minutes on ice. After homogenization, the suspensions were centrifuged at 3000 rpm for 10 minutes at 4°C. Supernatants were separated and stored at −80°C for further analysis of cytokines and other inflammatory mediators.[22]

The atopic status of study patients was evaluated by using the ImmunoCAP assay (Phadia, Uppsala, Sweden), which detects IgE antibodies against 6 mixtures of common aeroallergens (house dust mites, molds, trees, weeds, grass, and animal dander). Patients were considered atopic if the allergen-specific IgE level was greater than 3.51 kU/L. The diagnosis of asthma and aspirin sensitivity was performed by an allergist based on lung function and challenge tests. Lund-Mackay CT scores and Lund-Kennedy endoscopic scores were obtained before surgery and 6 months after surgery, respectively (Table I).

All patients provided written informed consent for study participation. This study was approved by the Internal Review Board of Seoul National University Hospital, Boramae Medical Center (no. 06-2012-109).

Immunohistochemistry

Paraffin sections were treated with 3% hydrogen peroxide (H_2O_2) and then incubated with primary antibodies and biotinylated secondary antibodies. Immune complexes were visualized with the Vectastatin ABC Kit (Vector Laboratories, Burlingame, Calif). The numbers of positive cells in epithelia, glands, and submucosa were counted in the densest tissue region in 5 high-power fields (hpfs; ×400 magnification) by 2 independent observers, and average values were scored. Detailed immunohistochemistry (IHC) procedures are described in the Methods section in this article's Online Repository at www.jacionline.org.

Quantitative real-time RT-PCR for inflammatory markers

The mRNA levels of IL-25, T-box transcription factor (T-bet), GATA3, RAR-related orphan receptor C (RORC), eosinophil cationic protein (ECP), TGF-β1, TGF-β2, and several cytokines and chemokines in human NP tissues, mouse nasal tissues, or both were evaluated by using semiquantitative real-time PCR analysis, as previously described.[23] Detailed semiquantitative real-time PCR conditions are described in the Methods section in this article's Online Repository.

ELISA for IL-25 and IL-17 receptor B in human tissue homogenates

IL-25 (R&D Systems, Minneapolis, Minn) and IL-17 receptor (IL-17R) B (R&D Systems) levels were measured with commercially available ELISA kits. The minimal detection limits for these kits are 62.5 and 156 pg/mL, respectively. All procedures followed the manufacturer's recommendations. Concentrations of IL-25 and IL-17RB in the tissue homogenate were normalized to the concentration of total protein. Detailed methods are described in the Methods section in this article's Online Repository.

Murine NP model

All animal experiments were approved by the Institutional Animal Care and Use Committee of Boramae Medical Center (No. 2013-0001) and performed under strict governmental and international guidelines on animal

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