ま と め

好酸球性副鼻腔炎は従来型の慢性副鼻腔炎と臨床的特徴が異なり，その相違はそれぞれの病態の違いを反映している．好酸球性副鼻腔炎の特徴である著明な好酸球性炎症には全身性のI型アレルギーの関与は必要ない．近年，ウイルスなどがToll-like receptorを介して好酸球性炎症を惹起するメカニズムも知られ，さらに上述したような真菌や細菌が局所的にIgE産生や好酸球性炎症を生じるメカニズムも報告されている．これらのどのメカニズムが主要であるかは好酸球性副鼻腔炎の個々の患者によってさまざまであろうが，これらの非特異的刺激は呼気流によって鼻腔内に到達するのであり，鼻腔内で直接的に呼気流に最も接する中鼻甲介が病変の主体になることは容易に推測される．さらに，非アトピー型喘息に高率に合併することからも，好酸球性副鼻腔炎の病態には全身性の要因(体質)の関与も考えられ，アラキドン酸カスケードの異常はその有力候補であろう．

好酸球性副鼻腔炎の鼻・副鼻腔粘膜や鼻茸組織は手術での採取が容易であり，それらを用いた病態研究の成果は非アトピー型喘息の病態解明にも寄与すると期待される．

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ORIGINAL ARTICLE

Clinical Epidemiological Study of 553 Patients with Chronic Rhinosinusitis in Japan

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ABSTRACT

Background: The relationship between chronic rhinosinusitis (CRS) and asthma has been known for a long time. However, no large studies on the relationship between CRS and lower airway diseases have been reported to date in Japan. Additionally, eosinophilic chronic rhinosinusitis (ECRS) in Japan is considered to be a subgroup of CRS with nasal polyps (CRSwNP) characterized by eosinophil-dominant inflammation. However, the diagnostic criteria of ECRS have not been established.

Methods: To investigate clinical and epidemiological features of patients with CRS from the aspect of their associations with lower airway diseases, 553 patients with CRS who visited one of six local university hospitals were examined and interviewed. Local eosinophilic infiltration was evaluated pathologically by exmining NPs.

Results: The prevalences of olfactory dysfunction (OD) in the patients with nasal polyps (NPs) and those without NPs were 57.0% and 13.7%, respectively (p < 0.0001). The prevalence of asthma in all patients was 23.1%. Furthermore, the prevalences of NPs and OD in the patients with asthma and those without asthma were 81.0% and 50.1% (p < 0.0001) and 64.2% and 35.7% (p < 0.0001), respectively. 97.4% of the patients with asthma had ≥15% mucosal eosinophils, and 87.9% of the patients without asthma had <15% mucosal eosinophilis.

Conclusions: Similar to the relationship between nasal allergy and asthma, CRSwNP may be applicable to the concept of "one airway, one disease".

KEY WORDS
asthma, chronic rhinosinusitis, eosinophil, nasal polyp, questionnaire survey

INTRODUCTION

Chronic rhinosinusitis (CRS) is a common disease in the otorhinolaryngology field in Japan. Previously, CRS mostly consisted of chronic inflammation caused by occlusion of the sinus ostia. Recently, however, CRS with nasal polyps (NPs), in which distinct eosinophilic infiltration can be observed, is rapidly increasing. This latter disease is called eosinophilic chronic rhinosinusitis (ECRS) in Japan.[1,2] In Europe and the United States, CRS characterized by eosinophilic inflammation is called chronic hyperplastic eosinophilic sinusitis (CHES),[3] eosinophilic mucin rhinosinusitis (EMRS)[4] or chronic hyperplastic rhinosinusitis with nasal polyposis (CHS/NP).[5] Each of these conditions can be considered to be similar to ECRS. Moreover, CRS has recently been divided into two subgroups: chronic rhinosinusitis with nasal polyps (CRSwNP) and chronic rhinosinusitis without nasal polyps (CRSsNP).[6] Ishitoya *et al.*[2] reported that the concept of ECRS in Japan would be applicable for CRSwNP in Europe and the United States.

The relationship between CRS and asthma has been known for a long time. However, it is not fully

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understood. An epidemiological study reported that 93% of patients with EMRS were associated with asthma and that 54% of these patients had aspirin-intolerant asthma (AIA).[4] Another study reported that 50% of CHS/NP patients were associated with asthma and that about 30-40% of these patients had AIA.[5] Thus, ECRS is an eosinophilic inflammation that can affect not only the nose and sinuses but also the bronchi, and can be considered to be an airway disease consistent with the concept of "one airway, one disease".

There have been epidemiological studies on upper airway diseases in Japan from the aspect of asthma.[7] However, from the aspect of CRS, no large studies on the relationship between CRS and lower airway diseases have been reported to date. In this study, we conducted a large-scale survey among multiple institutions (six university hospitals). The study focused on the clinical conditions of CRS and assessed the relationships with lower airway diseases.

METHODS

PATIENTS

Patients with CRS who visited one of six local university hospitals during 5 months from June 2005 to October 2005 were evaluated. Patients with CRS who visited during that period were randomly selected, and examined and interviewed for the items described below. A total of 553 patients were included, comprising 317 males, 229 females and 7 patients in whom no gender was specified. The mean age was 51.5 ± 18.8 years (range: 3-92 years) and the mean duration of CRS was 8.8 ± 13.2 years (range: 0.3-66 years).

The study was approved by the ethical committee of each institution, and informed written consent to the study protocol was obtained from all subjects.

CHRONIC RHINOSINUSITIS

The diagnostic criteria for CRS were defined by the clinical symptoms (nasal obstruction, discolored discharge (anterior/posterior nasal drip) or reduction/loss of smell) for more than 12 weeks, plain X-ray imaging and computed tomographic (CT) scanning. The patients were asked about the disease duration, whether they had an olfactory dysfunction (anosmia/hyposmia) and whether they had a history of surgery for sinus diseases. The presence or absence of an olfactory dysfunction was determined based on the patient's complaints. Plain X-ray examination, CT scanning and endoscopy were performed on all patients. The presence or absence of NPs was determined visually using an endoscope. Surgery was reserved for CRS patients with NPs or persistent cases after conservative medication for a few months. Local eosinophilic infiltration was evaluated pathologically using NPs removed during surgery. The NPs were stained with hematoxylin and eosin for detection of tissue eosinophilia. The percentage of eosinophils in each sample was calculated by dividing the number of eosinophils by the total number of inflammatory cells counted. We classified the degree of eosinophilic infiltration into the following three groups by comparing the percentages of eosinophils: mild (<15%); moderate (15-30%) and severe (≥30%). Patients with moderate to severe degrees of eosinophilic infiltration were considered to have the presence of eosinophilic infiltration. Our diagnostic criterion for ECRS is mucosal eosinophilia (≥15%). The procedures were carried out by experienced pathologists without knowledge of the other clinical parameters in each hospital.

LOWER AIRWAY DISEASES

Patients were asked if they had asthma that was still under treatment and about the disease duration. Among the patients with asthma, those who had experienced an attack induced by any non-steroidal anti-inflammatory drug were considered to have aspirin-intolerant asthma (AIA). Patients were also asked whether their asthma had been improved after endoscopic sinus surgery (ESS). Patients whose medication dose or attack frequency had been reduced were categorized into the improved group. The patients were also asked whether they had any chronic obstructive pulmonary diseases (COPD).

STATISTICAL ANALYSIS

The prevalences of NPs and an olfactory dysfunction, prevalences of lower airway diseases, prevalences of NPs and an olfactory dysfunction in patients with each lower airway disease, and surgery rates were evaluated. The chi-square test was used for statistical analyses of differences between groups and values of $p < 0.05$ were considered to indicate statistical significance. JMP software version 7.0.1 (SAS Institute Inc., Cary, NC, USA) was used for all analyses.

RESULTS

NASAL POLYPS AND OLFACTORY DYSFUNCTION IN ALL PATIENTS

Among all the 553 patients with CRS, 309 (55.9%) had NPs and 227 (41.0%) did not have NPs, while no findings were specified for 17 (3.1%). Furthermore, among the 553 patients with CRS, 210 (38.0%) had an olfactory dysfunction and 279 (50.5%) did not have an olfactory dysfunction, while no findings were specified for 64 (11.5%) (Table 1).

The relationship between NPs and olfactory dysfunction was also evaluated. An olfactory dysfunction was present in 176 of 309 (57.0%) patients with NPs, compared with 31 of 227 (13.7%) patients without NPs (Fig. 1). There was a significant difference in the prevalences of olfactory dysfunction between the two groups ($p < 0.0001$).

Table 1 Nasal polyps and olfactory dysfunction in 553 patients with chronic rhinosinusitis

	Present (%)	Absent (%)	Unknown (%)
Nasal polyps	309 (55.9)	227 (41.0)	17 (3.1)
Olfactory dysfunction	210 (38.0)	279 (50.5)	64 (11.5)

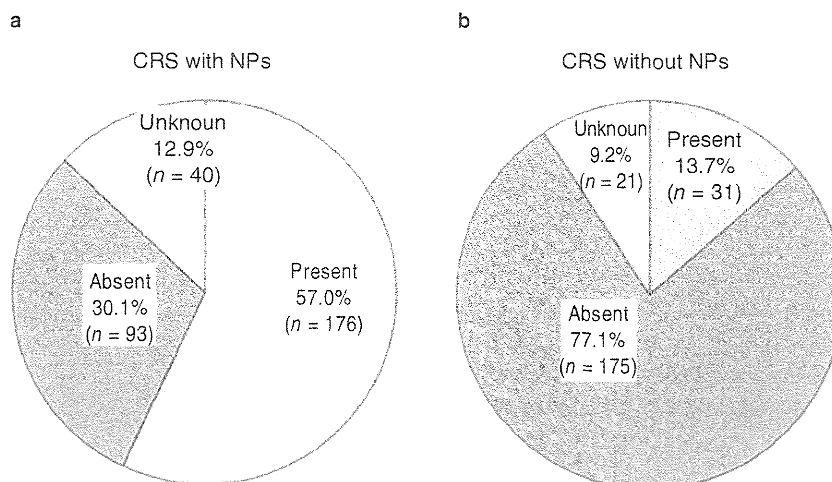

Fig. 1 Relationship between NPs and olfactory dysfunction. An olfactory dysfunction was reported in 176 of 309 (57.0%) patients with NPs (**a**), compared with 31 of 227 (13.7%) patients without NPs (**b**).

Table 2 Nasal polyps and olfactory dysfunction in 255 patients undergoing surgery

	Present (%)	Absent (%)	Unknown (%)
Nasal polyps	172 (67.5)	76 (29.8)	7 (2.7)
Olfactory dysfunction	121 (47.5)	117 (45.9)	17 (6.6)

NASAL POLYPS AND OLFACTORY DYSFUNCTION IN PATIENTS UNDERGOING SURGERY

Among the 553 patients with CRS, ESS was performed in 255 (46.1%). Among the 255 patients who underwent ESS, 172 (67.5%) had NPs and 76 (29.8%) did not have NPs, while no findings were specified for 7 (2.7%). Furthermore, among the 255 patients, 121 (47.5%) had an olfactory dysfunction and 117 (45.9%) did not have an olfactory dysfunction, while no findings were specified for 17 (6.6%) (Table 2).

PREVALENCES OF LOWER AIRWAY DISEASES IN ALL PATIENTS

Among the 553 patients with CRS, 128 (23.1%) had asthma (ATA in 99 [17.9%] and AIA in 29 [5.2%]) (Fig. 2). In addition, 15 patients had COPD (2.7%). The onset times of CRS and asthma were assessed. Among the 128 patients who had both CRS and asthma, the onset times of both diseases could be identified in 52 patients. Of these 52 patients, 15 (28.8%) developed CRS first, 23 (44.2%) developed asthma first and 14 (26.9%) developed both diseases simultaneously. There were no significant differences in the onset times between the two diseases.

PREVALENCES OF NASAL POLYPS AND OLFACTORY DYSFUNCTION IN PATIENTS WITH LOWER AIRWAY DISEASES

The prevalence of NPs was assessed for each lower airway disease. Overall, 76 of 97 (78.4%) patients with ATA, 26 of 29 (89.7%) patients with AIA and 7 of 13 (53.8%) patients with COPD had NPs. Among 400 patients without lower airway diseases, 200 (50.0%) had NPs. As expected, about 90% of patients in the AIA group and about 80% of patients in the ATA group had NPs. The prevalences were significantly higher in the ATA and AIA groups than in the group without lower airway diseases ($p < 0.0001$ for each group) (Fig. 3).

In the same way, the prevalence of patients with an olfactory dysfunction was assessed for each lower airway disease. Overall, 54 of 93 (58.1%) patients with

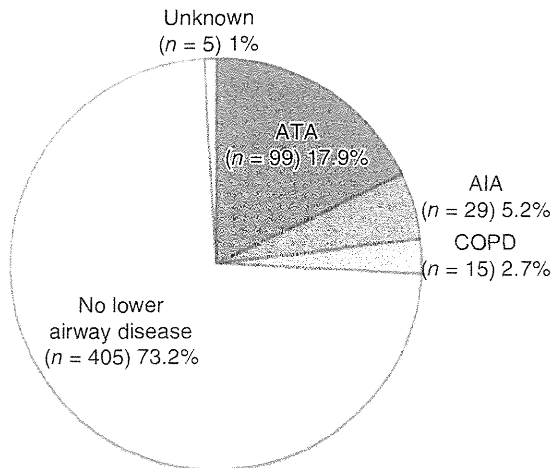

Fig. 2 Prevalences of lower airway diseases in 553 patients with chronic sinusitis. Among the 553 patients, 128 (23.1%) patients were associated with asthma, comprising 99 (17.9%) patients with ATA and 29 (5.2%) patients with AIA.

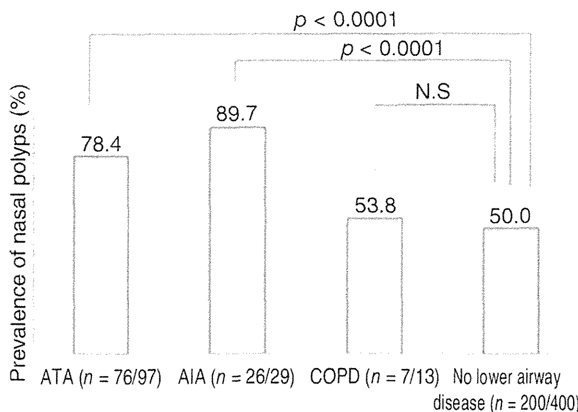

Fig. 3 Prevalences of NPs in patients with lower airway diseases. The prevalences of NPs are significantly higher in the ATA and AIA groups than in the group without lower airway diseases ($p < 0.0001$ for each group). N.S, not statistically.

ATA, 23 of 27 (85.2%) patients with AIA and 9 of 14 (64.3%) patients with COPD had an olfactory dysfunction. Among 359 patients without lower airway diseases, 124 (34.5%) patients had an olfactory dysfunction. The prevalences of patients with an olfactory dysfunction were significantly higher in the ATA and AIA groups than in the group without lower airway diseases ($p = 0.0002$ and $p < 0.0001$, respectively). In addition, the prevalence was significantly higher in the COPD group than in the group without lower airway diseases ($p = 0.0311$) (Fig. 4).

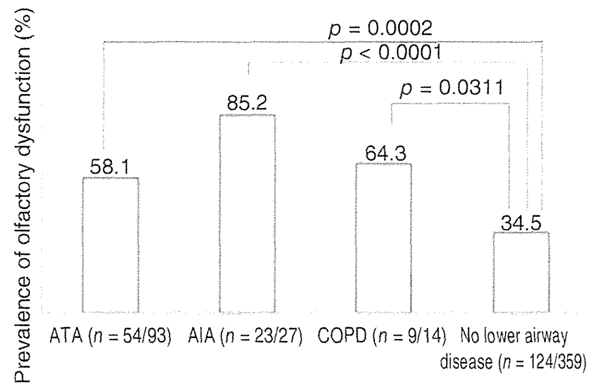

Fig. 4 Prevalences of olfactory dysfunction in patients with lower airway diseases. The prevalences of olfactory dysfunction are significantly higher in the ATA, AIA and COPD groups than in the group without lower airway diseases ($p = 0.0002$, $p < 0.0001$ and $p = 0.0311$, respectively).

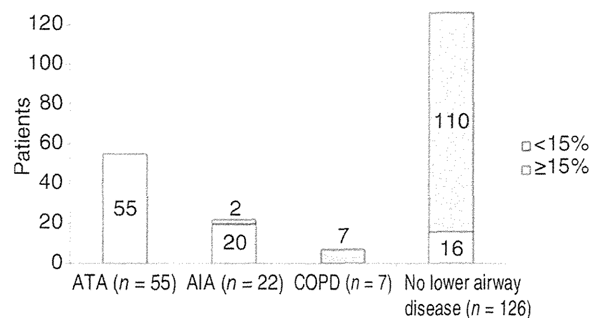

Fig. 5 Eosinophilic infiltration in patients with lower airway diseases. Among 210 patients who assessed for eosinophilic infiltration, 55 of 55 (100%) patients with ATA, 20 of 22 (90.9%) patients with AIA and 16 of 133 (12.0%) patients without asthma had ≥15% eosinophils.

EOSINOPHILIC INFILTRATION

Among 255 patients who underwent ESS, 91 (35.7%) had ≥15% eosinophils and 119 (46.7%) had <15% eosinophils, while no findings were specified for 45 (17.6%). Among 210 patients who assessed for eosinophilic infiltration, 55 of 55 (100%) patients with ATA, 20 of 22 (90.9%) patients with AIA and 16 of 133 (12.0%) patients without asthma had ≥15% eosinophils (Fig. 5).

In addition, among 198 patients in whom the presence or absence of olfactory dysfunction and eosinophilic infiltration could be determined, the relationship between olfactory dysfunction and eosinophilic infiltration was assessed. Among the 198 patients, 99 (50.0%) had an olfactory dysfunction, 90 (45.5%) had eosinophilic infiltration and 71 (35.9%) had both an olfactory dysfunction and eosinophilic infiltration (Table 3).

Table 3 Relationship between olfactory dysfunction and eosinophilic infiltration in 198 patients in whom the presence or absence of an olfactory dysfunction and eosinophilic infiltration could be confirmed

		Olfactory dysfunction	
		+	-
Eosinophilic infiltration (≥15%)	+	71	19
	-	28	80

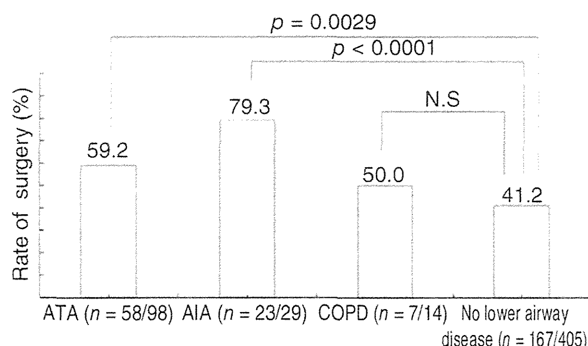

Fig. 6 Surgery rates in patients with lower airway diseases. The rates of surgery are significantly higher in the ATA and AIA groups than in the group without lower airway diseases (p = 0.0029 and p < 0.0001, respectively). N.S, not statistically.

SURGERY RATES IN PATIENTS WITH LOWER AIRWAY DISEASES

Overall, 58 of 98 (59.2%) patients with ATA, 23 of 29 (79.3%) patients with AIA and 7 of 14 (50.0%) patients with COPD underwent ESS. In contrast, among 405 patients without lower airway diseases, 167 (41.2%) underwent ESS. The percentages of patients undergoing ESS were significantly higher in the ATA and AIA groups than in the group without lower airway diseases (p = 0.0029 and p < 0.0001, respectively) (Fig. 6).

IMPROVEMENT IN ASTHMA AFTER SURGERY

Sixty-four patients with asthma in whom changes in asthma after surgery could be identified were evaluated. Among these 64 patients, 47 (73.4%) showed an improvement and 17 (26.6%) did not show any improvement. No patients showed exacerbation of asthma.

DISCUSSION

CRS is a common disease in the otorhinolaryngology field. In recent years, with a decrease in CRS caused by occlusion of the sinus ostia, which previously accounted for the majority of sinusitis cases, sinusitis showing distinct infiltration of activated eosinophils in the sinus mucosa has been increasing. In 2006, CRS has been divided into two subgroups: CRSwNP and CRSsNP.[6] ECRS in Japan is considered to be a subgroup of CRSwNP in Europe and the United States.[2] ECRS is characterized by adult onset, bilateral lesions affecting the ethmoidal sinus rather than the maxillary sinus, frequent olfactory disturbance and resistance to treatment. Given the fact that systemic steroid administration is effective, involvement of allergy is suspected, although it remains unclear whether a type I allergy is involved. Long-term low-dose macrolide therapy is reported to be useful in postoperative treatment for CRS[8] and is widely used in clinical practice. However, in cases involving eosinophilic inflammation, therapies with 14-membered ring macrolide antibiotics are not effective and exacerbation is frequently observed. In addition, it has been reported that the exacerbation may be related to the lower airways, and that it has a high complication rate for asthma including AIA.[4,5]

Because awareness of the association between allergic rhinitis as an upper airway disease and asthma has been growing, international guidelines for allergic rhinitis, designated Allergic Rhinitis and its Impact on Asthma (ARIA), were proposed through a joint project by the WHO and the International Association of Allergy and Clinical Immunology in 2001 and revised in 2008.[9,10] In consideration of the relationship between CRS and asthma, we wondered whether this relationship was consistent with the concept of "one airway, one disease", similar to the relationship between allergic rhinitis and asthma. The prevalences of asthma in patients with CRS in previous reports were 23-50%.[11-13] In this study, 23% of patients with CRS were associated with asthma. These figures are higher than the asthma prevalence of 5% in the general population.[14] In addition, the prevalence of asthma in patients with CRS caused by occlusion of the sinus ostia is considered to comprise only a small percentage.[15] From an epidemiological viewpoint, it indicated that CRS have a relationship with asthma. Moreover, the prevalence of NPs in patients with asthma was significantly higher than that in patients without asthma, indicating that CRSwNP have a close relationship with asthma.

Focusing on eosinophils, Jankowski *et al.*[16] studied nasal polyposis histologically and reported that 102 of 123 (82.9%) patients with nasal polyposis showed >20% eosinophils and all 25 patients in a control group for CRS showed <10% eosinophils. In Japan, histopathological diagnostic criteria for ECRS have not been established. When we defined ≥15% eosinophils as the criterion for ECRS, almost all CRS patients with asthma met our criterion. In addition, 91 (35.7%) of 255 patients who underwent ESS also met our criterion. From histological viewpoint, it is suggested that eosinophilic infiltration are highly correlated with CRSwNP and asthma. Therefore, the concepts of

CRSwNP and CRSsNP need to be separated.

Olfactory dysfunction is one of only four signs and symptoms included in the diagnostic criteria for CRS, and it affects about 60% of CRS patients.[17] Classically, olfactory dysfunction in CRS was caused by mechanical obstruction of the olfactory cleft by physical obstruction of the nasal airways, nasal polyposis, edema, and secretions.[18] However, Kern reported that CRS patients had evidence of direct inflammation of the neuroepithelium and that the degree of inflammatory changes in the neuroepithelium was related to the severity of olfactory dysfunction.[19] In the present study, the prevalences of NPs and olfactory dysfunction were significantly higher in asthmatic groups than in the group without asthma. It seems that olfactory dysfunction by CRS is caused by both conductive and sensorineural process, and that the inflammatory response and the effect to the respiratory epithelium are more severe in CRS patients with asthma than in the patients without asthma.

Interestingly, surgery for CRS improved the asthma symptoms. There are several previous papers reporting that ESS in patients with asthma resulted in improvement of asthma symptoms, reduction in medication doses and less frequent attacks.[20-23] This study was based on a questionnaire survey rather than physical exam, however, 73.4% of patients showed improvement in asthma after ESS, which strongly suggests a relationship between the two diseases in terms of treatment.

Based on the results of this study, the relationship between ECRS and asthma may be applicable to the concept of "one airway, one disease".

CONFLICT OF INTEREST

No potential conflict of interest was disclosed.

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ORIGINAL ARTICLE

Interleukin-19 Downregulates Interleukin-4-Induced Eotaxin Production in Human Nasal Fibroblasts

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ABSTRACT

Background: Interleukin-19 (IL-19), a member of the IL-10 family, is characterized as the cytokine suppressing the release and function of several proinflammatory cytokines. For regulation of local reaction in allergic rhinitis (AR), IL-19 might play an especially important role.

Methods: We examined effects of IL-19 on IL-4-induced eotaxin production by human nasal fibroblasts. Early receptor-mediated events (expression of the suppressors of cytokine signaling (SOCS) and phosphorylation of signal transducer and activator of transcription 6 [STAT6]) by IL-19 was examined. Knockdown methods by RNAi were administered to investigate the involvement of those signal transductions.

Results: Pretreatment with IL-19 downregulates IL-4-induced eotaxin production, but not interferon-γ (IFN-γ)-induced RANTES. Pretreatment with IL-19 suppressed the IL-4-induced STAT6 phosphorylation. The IL-19 induced SOCS-1, but not SOCS-3 or SOCS-5. The SOCS-1 knockdown by RNAi diminished pretreatment with IL-19-induced down-regulation of eotaxin production.

Conclusions: These results suggest that IL-19 down-regulates IL-4-induced eotaxin production via SOCS-1 in human nasal fibroblasts. In non-hematopoietic cells in AR, IL-19 might be an immunosuppressive factor.

KEY WORDS

eotaxin, human nasal fibroblast, Interluekin-19, SOCS-1, STAT6

INTRODUCTION

As an important immunoregulatory cytokine, IL-10 is known to have multiple biologic effects on different cell types. The IL-10 family includes IL-19, IL-20, IL-22, IL-24, and IL-26.[1-3] In fact, IL-19 is detected by human monocytes, B cells, and T cells.[3] Inflammatory stimulation such as that by lipopolysaccharide (LPS) or GM-CSF treatment induces IL-19 mRNA by monocytes. Pre-priming monocytes with IL-4 enhances the induction of IL-19 by LPS-treatment, although pre-priming with IFN-γ apparently prevents LPS-induced IL-19 expression.[4] Furthermore, in whole peripheral blood mononuclear cells cultured with Con-A, IL-19 up-regulated IL-4 and down-regulated IFN-γ dose-dependently.[4] The serum levels of IL-19 in children with atopic asthma were twice those of healthy children.[5] The *in vitro* treatment of IL-19 induced IL-4, IL-5, IL-10, and IL-13 production by activated T cells.[5] Long-term exposure of naïve T cells to IL-19 down-regulated the differentiation to IFN-γ-producing T cells, but up-regulated differentiation to IL-4 and IL-13 producing T cells.[6] These results suggest that IL-19 positively contributes to Th2 response.

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Reportedly, nasal fibroblasts are not passive players in the immune system.[7] Fibroblasts, long considered mainly as constituting a physical barrier, have recently been reported as important modulators of local inflammation because of their capacity to release various pro-inflammatory mediators including eotaxin, RANTES, thymus, and activation-regulated chemokine (TARC), GM-CSF, and IL-8.[8-10] Eotaxin and RANTES are implicated in the recruitment and enhanced survival of eosinophils[11,12]; TARC causes selective migration of Th2 cells.[10] We showed previously that pro-inflammatory cytokines and IFN-γ induced production of RANTES and that IL-4 induced eotaxin by nasal fibroblasts.[13]

Recently it was reported that vascular smooth muscle cells[14] and airway epithelial cells[15] express IL-19, although IL-19 expression had been ascribed to be restricted to immune cells. Nevertheless, the influence of IL-19 on the structural cells (ex fibroblasts, epithelial cells) remains unclear.

In this study, we investigated the effect of IL-19 on chemokine production by human nasal fibroblasts. Results show that IL-19 inhibited IL-4-induced eotaxin production by fibroblasts, which was unexpected because IL-19 has been shown to be an inducer of Th2 cytokine (Th2 immunity).[3-6] Actually, the Th2/Th1 shift induced by IL-19 might depend on the cell type. Fulfillment of IL-19 function requires the induction of suppressors of cytokine signaling-1 (SOCS-1) in fibroblasts.

METHODS

REAGENTS

Recombinant human IL-4, IL-19, and IFN-γ were obtained from PeproTech EC Ltd. (London, UK). The eotaxin and RANTES kits were purchased from Biosource International Inc. (Camarillo, CA, USA).

HUMAN NASAL MUCOSA-DERIVED FIBROBLAST CELL CULTURE AND STIMULATION

Nasal mucosa of the inferior turbinate were obtained from patients with allergic rhinitis (AR) when they underwent nasal surgery, as described previously.[9,13] Nasal specimens were cultured in 10 cm dishes containing medium (RPMI 1640; Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% heat-inactivated FCS (Gibco, Grand Island, NY, USA), 0.29 mg/ml glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin, at 37℃ in 5% CO_2 and humidified air. Nasal fragments were removed and the first passage was performed. After 3-4 weeks, nasal-mucosa-derived fibroblast cell lines were established. The cells were used at passage numbers 3-5. Using cytokeratin and vimentin markers, epithelial cells were confirmed not to be contaminated by immunohistochemical examination. The cells were then placed in a 24-well flat-bottomed tissue culture plate at an initial density of 1×10^5 cells/well for chemokine production or a 10 cm dish for Western blot and RT-PCR.

CYTOKINE AND CHEMOKINE ASSAY

The cells were cultured in the presence of cytokines (IL-4, IFN-γ, IL-19) for appropriate periods; then culture supernatants were harvested. Amounts of chemokines in the cell culture supernatant were measured using commercially available ELISA kits. Measurements were performed according to the manufacturer's directions. All samples were assayed in duplicate.

RT-PCR ANALYSIS

Total RNA was extracted using a total RNA isolation NucleoSpin™ RNA II Kit (Macherey-Nagel, Duren, Germany). The reverse transcription reaction was performed using Taqman RT Reagents (Applied Biosystems Japan, Tokyo, Japan) with random hexamer primers. Using 2 µg of total RNA, first-strand cDNA was synthesized using a first-strand cDNA synthesis kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA). Then 1 µl of the resulting first-strand cDNA was used for each PCR.[16] The following primers for human IL-20R1 and IL-20R2 were used in the reactions: IL-20R1, 5'-TCAAACAGAACGTGGTCCCA GTG-3' (nucleotides 1001-23) and 5'-TCCGAGATATT GAGGGTGATAAAG-3' (nucleotides 1369-92), IL-20R 2, 5'-GCTGGTGCTCACTCACTGAAGGT-3' (nucleotides 509-31), and 5'-TCTGTCTGGCTGAAGGCGCT GTA-3' (nucleotides 892-914). The reaction mixture in a final volume of 25 µl consisted of 1 × Taq DNA polymerase buffer, 0.2 mM dNTP, 1.5 mM MgCl2, 0.5 µM of each primer, and 2.5 U of Taq DNA polymerase (Amersham Pharmacia Biotech Inc.) according to the instructions for the Taq DNA polymerase. Pre-amplification denaturation was performed at 94℃ for 5 min and amplification was conducted for 35 cycles. The respective cycles were 94℃ for 1 min, 65℃ for 1 min, and 72℃ for 1 min. The final extension step was performed for 7 min at 72°C. Samples (10 µl) of the PCR products were analyzed on 2% agarose gel in 1 × Tris-acetate-EDTA (TAE) buffer; bands were visualized using ethidium bromide staining.

REAL TIME PCR

Total RNA was extracted and the reverse transcription reaction was performed as described above. The amplifications of SOCS-1, SOCS-3, SOCS-5, and β2-microlobulin-cDNA were performed in a MicroAmp optical 96-well reaction plate (Applied Biosystems Japan). All TaqMan probe/primer combinations used for this study were TaqMan Gene Expression Assay products purchased from Applied Biosystems. Because it is convenient to assay and because it is highly expressed, β2-microglobulin was chosen as the reference housekeeping gene. Furthermore, to select the housekeeping gene, we evaluated it using a TaqMan Human Endogenous Control Plate, which is

most suitable. TaqMan PCR was performed in a 20 µl volume using TaqMan Universal PCR master mix (Applied Biosystems Japan). The reaction was performed using a sequence detection system (ABI Prism 7000; Applied Biosystems Japan). Reaction mixtures were pre-incubated for 2 min at 50℃. The PCR program was 10 min of Taq Gold activation at 95℃, followed by 40 cycles of 15 s at 95℃ and 1 min at 60℃ (maximum ramping speed between temperatures). Human cDNA equivalent to 50 ng of total RNA from each sample was assayed in each tube.

The threshold cycle number (Ct) was determined using sequence detector software (ver. 1.1: Applied Biosystems Japan) and transformed using comparative Ct methods, as described by the manufacturer, with β2-microglobulin as the calibrator gene.

RNAi AGAINST IL-20R2 AND SOCS-1

Human IL-20R2 siRNA (5'-GAUGGCUUCCACCUGG UUA TT-3) and a nonspecific scrambled siRNA (5'-A GUUCUGGUCGCCGAUCUA TT-3') were purchased from Takara Bio Inc. (Otsu, Japan). Human SOCS-1 siRNA (5'-GGGUCUCUGGCUUUAUUUU TT-3') was purchased from Applied Biosystems Japan. Nasal fibroblast cells were transiently transfected with 1.3 µmol/L of specific siRNA targeting human IL-20R2, SOCS-1, or nonspecific siRNA for 2 hr using a Trans IT-TKO (Mirus Bio LLC, Madison, WI, USA) system according to the manufacturer's instructions. The medium was replaced with conditioning medium for an additional 24 hr.

WESTERN BLOT ANALYSIS

Human nasal fibroblasts were washed twice with ice-cold PBS and collected by scraping, then centrifuged and pelleted at 4℃. The nasal fibroblasts were homogenized in lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 1 mM PMSF, 0.5 mM EDTA, 0.6 µM leupeptin, 2 µM pepstatin A, and 1 mM PMSF] by pipetting and sonication. Protein concentrations were measured in all experiments using the BioRad Protein Assay Kit (Bio-Rad Laboratories Inc., Hercules, CA, USA). Lysates were centrifuged at 10000 rpm for 10 min at 4℃; the supernatants were used for immunoblotting. The supernatants were added to a twofold volume of sample buffer [95% Laemmli sample buffer (Bio-Rad Laboratories Inc.) and 5% 2-mercaptoethanol]. After heating at 95℃ for 5 min, the samples were electrophoresed. Proteins were transferred electrophoretically onto polyvinylidene fluoride (PVDF) membranes (Amersham Hybond-P, GE Healthcare, Tokyo, Japan). The blotted membranes were then rinsed with 5% non-fat dry milk diluted in PBS containing 0.1% Tween 20 for 60 min at room temperature. They were incubated overnight at 4℃ with the anti-STAT6 and anti-phospho-STAT6 polyclonal antibody (Ab) (1/1000 in 5% BSA in TBST) (Cell Signaling Technologies, St. Louis, MO, USA). After being washed, the membranes were treated with HRP-conjugated anti-mouse immunoglobulin (Ig) Ab or HRP anti-rabbit Ig Ab (Dako, Carpinteria, CA, USA) for 60 min at room temperature. The blot was washed again; then it was developed using the ECL plus Western blot detection reagents (Amersham Pharmacia Biotech Inc.).

IMMUNOHISTOCHEMICAL STAINING

Nasal specimens from patients with AR were obtained by turbinate surgery. Paraffin-embedded blocks were sliced into 4-µm-thick sections. The sections were deparaffinized with xylene, dehydrated in ethanol, and put into methanol containing 0.3% H_2O_2 for 15 min to block endogenous peroxidase activity. The sections were then incubated with normal bovine serum for 15 min at room temperature and treated overnight at 4℃ with anti-IL-19 monoclonal antibody (Santa Cruz Biotec Inc., CA, USA). The sections were then washed with PBS and incubated with a cocktail of peroxidase-labeled polymer conjugated to goat anti-mouse IgG antibody and goat anti-rabbit IgG antibody at room temperature for 1 h. Finally, the sections were incubated in PBS containing 0.03% DAB and 0.01% H_2O_2. All slides were lightly counterstained with Mayer's hematoxylin.

DATA AND STATISTICAL ANALYSES

Data in the text and figure legends are expressed as the mean ± SEM of observations. Statistical analyses were Wilcoxon signed-rank tests used to assess the difference in chemokine production levels. Computers with Microsoft Windows OS with software (Statview; Abacus Concepts Inc., Berkeley, CA, USA) were used for all statistical analyses.

RESULTS

HUMAN NASAL FIBROBLASTS EXPRESS IL-20 R1 AND IL-20R2

We first set out to determine the expression of IL-20R1 and IL-20R2 because IL-19 binds two distinct receptor complexes consisting of the IL-20R1 and IL-20R2 chains.[17-19] The expression of mRNA components for IL-20R1 (392 bp) and IL-20R2 (406 bp) were determined in all nasal fibroblasts (n = 6) using RT-PCR (Fig. 1a). These bands were fully sequenced and identified as IL-20R1 and IL-20R2 (data not shown).

NASAL EPITHELIA EXPRESS IL-19

Ten formalin-fixed paraffin-embedded nasal mucosa specimens were stained using ABC methods with anti-human IL-19 monoclonal antibody. The cytoplasm and plasma membranes of epithelial cells were positively stained (Fig. 1b), as were infiltrated cells in the lamina propria, indicating the possible production of IL-19 by inflammatory cells.

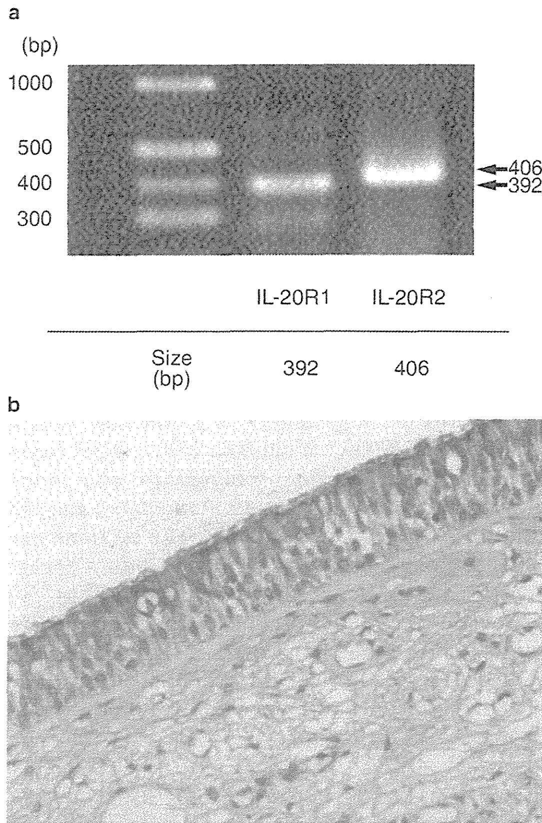

Fig. 1 (**a**) Expression of components of IL-19 receptors in human nasal fibroblast was determined using RT-PCR. IL-19 binds to IL-20R1 and IL-20R2. All nasal fibroblasts (n = 6) express both IL-20R1 and IL-20R2. (**b**) Immunohistochemical staining of IL-19 in nasal mucosa showed epithelial cells and infiltrated cells positively.

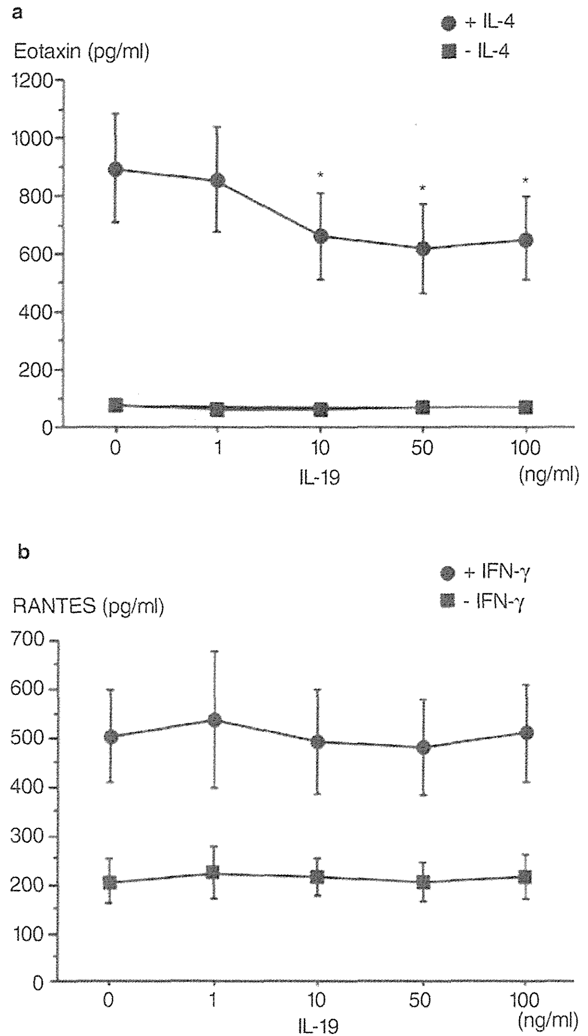

Fig. 2 IL-4 induced eotaxin, and IFN-γ induced RANTES by human nasal fibroblasts. Nasal fibroblast cells were pretreated with IL-19 (0, 1, 10, 50, 100 ng/ml) for 24 hr and stimulated with IL-4 (10 ng/ml) (**a**) or IFN-γ (30 ng/ml) (**b**) for 24 hr. (**a**) IL-19 inhibited IL-4-induced eotaxin production (n = 6). * p < 0.05 compared to eotaxin value of the supernatant in the presence of IL-4 without IL-19. (**b**) IL-19 did not act on IFN-γ-induced RANTES production (n = 6).

IL-19 SUPPRESSED EOTAXIN PRODUCTION BY IL-4 IN HUMAN NASAL FIBROBLASTS

Nasal fibroblasts can release various chemokines. Actually, IL-4, a representative Th2 cytokine, induced eotaxin in human nasal fibroblasts[13] although IFN-γ, Th1 cytokine induced RANTES.[20] Simultaneous stimulation with IL-4 (10 ng/ml) and IL-19 (50 ng/ml) for 24 hr induced equal amounts of eotaxin production to that by IL-4 alone from nasal fibroblasts (IL-19 + IL-4, 870 ± 205 pg/ml vs. IL-4, 822 ± 212 pg/ml, mean ± SE). Eotaxin production by simultaneous stimulation with IL-4 and IL-19 for 48 hr was not different from that obtained with IL-4 alone (data not shown).

Nasal fibroblasts were pretreated with each concentration of IL-19 for 24 hr and were then stimulated with IL-4 (10 ng/ml) for 24 hr. Significantly enhanced inhibition of eotaxin production was observed with 10 ng/ml of IL-19 (IL-19 + IL-4, 618 ± 153 pg/ml vs. IL-4, 896 ± 191 pg/ml, p < 0.05) (Fig. 2a). The suppression of eotaxin production reached a plateau from stimulation with 10 ng/ml of IL-19. The time course of IL-19 effects was determined. Inhibition of eotaxin production by IL-19 reached a plateau at 24 hr of pretreatment (data not shown). No difference in cell viability was found between IL-4 alone and IL-4 + IL-19. The nasal fibroblast shape was not altered by treatment with IL-19 (data not shown). We inferred that the optimal pretreatment time and concentration were, respectively, 24 hr and 10 ng/ml of IL-19. Pretreatment of IL-19 was critical for the inhibition of eotaxin production in nasal fibroblasts.

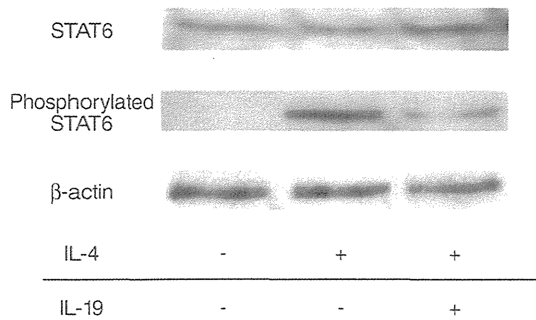

Fig. 3 IL-19 suppresses IL-4-induced STAT6 phosphorylation. Nasal fibroblasts were cultured in medium for 26 hr (lane 1), or with IL-4 (10 ng/ml) for 2 hr after incubation in medium for 24 hr (lane 2), or with IL-4 (10 ng/ml) for 2 hr after pretreatment of IL-19 (10 ng/ml) for 24 hr (lane 3). Samples were blotted with STAT6 Ab and phosphorylated STAT6 Ab.

The TNF-α enhanced eotaxin production by IL-4 in fibroblasts. The addition of TNF-α (50 ng/ml) to IL-4 enhanced eotaxin production (2537 ± 503 pg/ml) three-fold. Pretreatment of IL-19 (10 ng/ml) also inhibited TNF-α + IL-4-induced eotaxin production (1839 ± 330 pg/ml, $p < 0.05$ compared to TNF-α + IL-4). The inhibition rate of eotaxin production by IL-19 + TNF-α + IL-4 was not different from that by IL-19 + IL-4 (31% vs. 28%).

Nasal fibroblast released RANTES by IFN-γ (30 ng/ml) stimulation. Pretreatment of IL-19 (10 ng/ml) had no effect on RANTES production in nasal fibroblasts (IL-19 + IFN-γ, 491 ± 106 pg/ml vs. IFN-γ, 505 ± 95 pg/ml) (Fig. 2b). Although the concentration of IL-19 was changed, no effect of IL-19 on RANTES production was found. Simultaneous stimulation with IFN-γ and IL-19 had no effect on RANTES production (data not shown).

IL-19 SUPPRESSED IL-4-INDUCED STAT6 PHOSPHORYLATION

Results of previous studies show that the signal transducer and activator of transcription 6 (STAT6) was necessary for IL-4-induced eotaxin production.[21] We investigated the effect of IL-19 on tyrosine phosphorylation of STAT6. No phosphorylation of STAT6 was observed in unstimulated fibroblasts. When nasal fibroblasts were stimulated with IL-4 for 2 hr, tyrosine phosphorylation of STAT6 was induced (Fig. 3). However, pretreatment with IL-19 for 24 hr before stimulation with IL-4 reduced the amount of phosphorylated STAT6 in nasal fibroblasts.

IL-19 INDUCED SOCS-1 IN NASAL FIBROBLASTS

We tested next whether IL-19 might regulate early receptor-mediated events: expression of the SOCS regulatory proteins. The SOCS proteins–SOCS-1,[22] SOCS-3,[23] and SOCS-5[24] reportedly inhibit the IL-4/STAT6 signal transduction pathway. For that reason, we used real-time PCR to investigate whether expression of SOCS-1, SOCS-3, and SOCS-5 were induced by pretreatment with IL-19 for 24 hr in nasal fibroblasts. Treatment with IL-19 (10 ng/ml) for at least 2 hr significantly up-regulated the mRNA for SOCS-1 (3.0-fold compared to unstimulated cells, $p < 0.05$; Fig. 4a). The high level of mRNA for SOCS-1 continued to 24 hr after IL-19 stimulation. However, respective mRNA expressions of SOCS-3 and SOCS-5 were not enhanced by IL-19 treatment (Fig. 4b, c).

IL-19 HAD NO ENHANCEMENT OF SOCS-1 WHEN IL-20R2 WAS KNOCKED DOWN BY RNAi

To confirm that IL-19 enhanced SOCS-1 mRNA, IL-20R2 was changed artificially using RNAi method. Nasal fibroblasts were treated with RNAi according to the manufacturer's instructions. For nasal fibroblasts, the RNAi knockdown caused 49.3% reduction of IL-20R2 mRNA using real-time PCR.

Pretreatment with IL-19 enhanced the expression of SOCS-1 mRNA in the control siRNA-transfected cells (Fig. 5). However, the transfection of IL-20R2 siRNA abolished IL-19-enhanced SOCS-1 mRNA expression (Fig. 5, lane 4). No significant difference in cell viability was found among controlled fibroblasts and transfected fibroblasts with control RNAi or IL-20 R2 RNAi (data not shown). Eotaxin production was reversely correlated with SOCS-1 mRNA expression. Transfection of IL-20R2 siRNA to nasal fibroblasts increased IL-4-induced eotaxin production in the presence of IL-19 compared to fibroblasts treated with control siRNA (IL-20R2 RNAi, 476.2.1 ± 75.5 pg/ml vs. control RNAi, 342.0 ± 70.2 pg/ml, $p < 0.05$).

IL-19 SHOWED NO SUPPRESSION OF IL-4-INDUCED EOTAXIN PRODUCTION WHEN SOCS-1 WAS KNOCKED DOWN BY RNAi

Nasal fibroblasts were transfected with SOCS-1 siRNA to examine direct effects of SOCS-1 on suppression of IL-4-induced eotaxin production by IL-19. For nasal fibroblasts, RNAi knockdown caused 41.2% reduction of SOCS-1 mRNA by real time PCR. The transfection of SOCS-1 siRNA showed no enhancement of IL-4-induced eotaxin compared to that of the control siRNA in the absence of IL-19 (SOCS-1 RNAi, 564.0 ± 68.6 pg/ml vs. control RNAi, 523.4 ± 60.4 pg/ml, Fig. 6). Pretreatment with IL-19 reduced IL-4-induced eotaxin in the control siRNA transfected cells (381.0 ± 30.8 pg/ml, $p < 0.05$ compared to that in the absence of IL-19). However, pretreatment with IL-19 in the SOCS-1 siRNA transfected cells showed no suppression of IL-4-induced eotaxin production (540.6 ± 58.4 pg/ml). Cell viability and form in SOCS-1 siRNA transfected cells were not different from those in control siRNA transfected cells (data not shown).

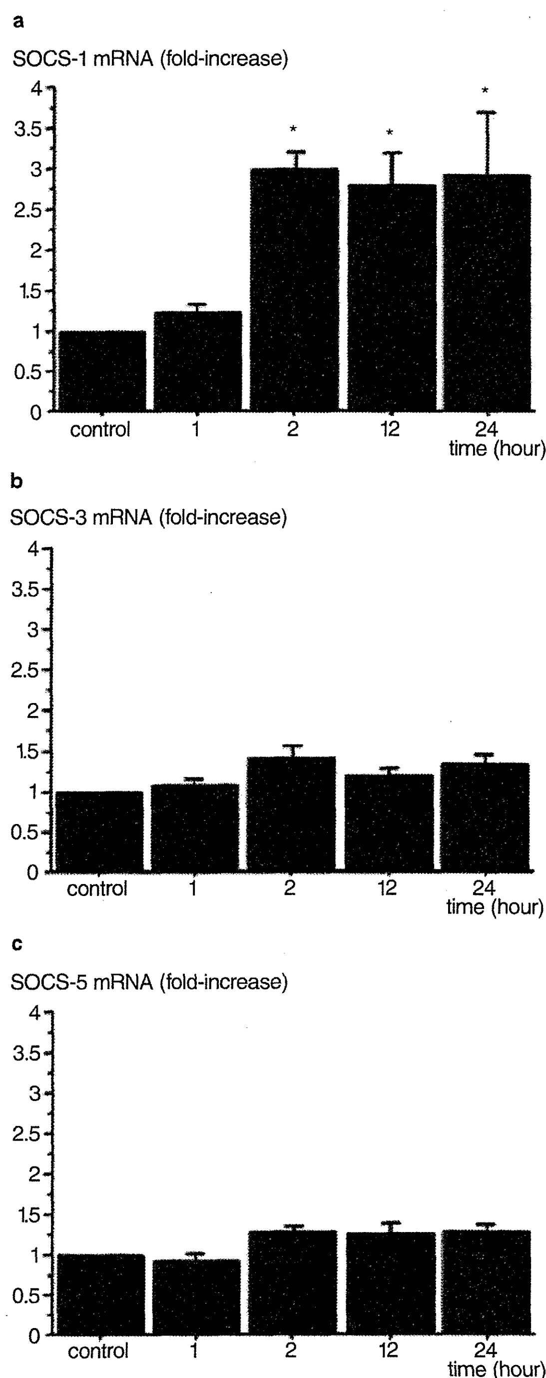

Fig. 4 IL-19 induced SOCS-1 (**a**), but not SOCS-3 (**b**) or SOCS-5 (**c**) by nasal fibroblasts. Nasal fibroblasts were cultured with or without IL-19 (10 ng/ml) each time. Respective expressions of SOCS-1, SOCS-3, and SOCS-5 in nasal fibroblasts were assayed using real-time PCR. Reactions were performed in three wells. The results are expressed relative to expression levels of β2-microglobulin. Data are presented as the mean ± SEM ($n = 9$). * $p < 0.05$ compared to control.

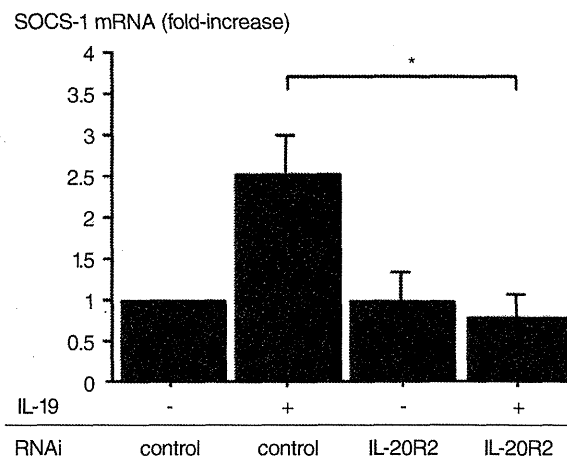

Fig. 5 Knockdown of IL-20R2 by RNAi abolished IL-19-induced SOCS-1 expression. Nasal fibroblasts were transfected by IL-20R2 siRNA or control siRNA. Then SOCS-1 mRNA was assayed using real-time PCR. Reactions were performed in three wells. The results are expressed relative to expression levels of β2-microglobulin. Data are presented as the mean ± SEM ($n = 5$). Knockdown of IL-20R2 by RNAi reduced SOCS-1 mRNA expression to the level of the control level. * $p < 0.05$.

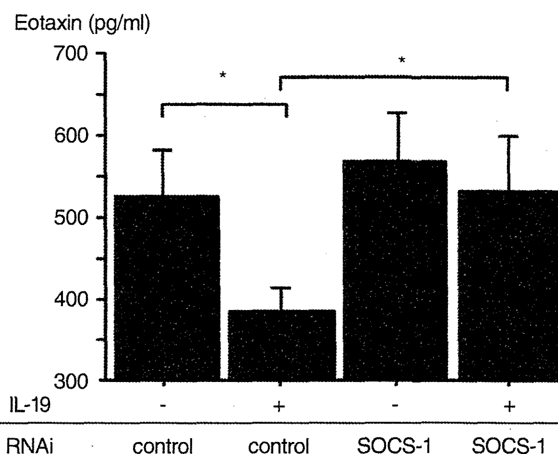

Fig. 6 Knockdown of SOCS-1 by RNAi abolished the suppression of IL-4-induced eotaxin production by IL-19. Nasal fibroblasts were transfected with SOCS-1 siRNA or control siRNA. Concentration of eotaxin in the supernatant of the culture system was assayed using ELISA. * $p < 0.05$.

DISCUSSION

In this study, we demonstrated that IL-19 inhibited IL-4-induced eotaxin production from human nasal fibroblasts, but not IFN-γ-induced RANTES. In fact, IL-19 induced SOCS-1 signaling, and the SOCS-1 signal

suppressed IL-4-induced tyrosine phosphorylation of STAT6 in nasal fibroblasts. The SOCS-3 and SOCS-5 mRNA were not induced by IL-19. These results suggest that IL-19 played a suppressive role of eosinophil-induced inflammation via suppression of eotaxin production in nasal fibroblasts of patients with AR.

The functions of many interleukins are elaborated by activation of intracellular signaling cascades involving the SOCS and STAT family of signaling proteins. Six SOCS family members exert their inhibitory effects by binding to tyrosine phosphorylated residues on signaling intermediates, protein kinases, and receptor chains, resulting in the attenuation of signaling. Then SOCS-1 and SOCS-3 inhibited IL-4-induced secretion of eotaxin in HEK293 cells.[25] In fact, IL-4-induced STAT6 activation was inhibited profoundly by 24 hr pretreatment with interferon (IFN)-γ in human primary airway epithelial cell cultures because IFN-γ pretreatment induced both SOCS-1 and SOCS-3.[26] Another group demonstrated that IFN-γ-induced SOCS-1 regulated STAT6-dependent eotaxin production triggered by IL-4 and TNF-α in mouse embryonic fibroblast from SOCS-1 knockout mice.[27] Our data, showing that IL-19-induced SOCS-1 inhibited eotaxin production from human nasal fibroblast via suppression of IL-4-induced STAT6, are consistent with evidence reported in the literature.

As the IL-19-inducible inhibitory signal transduction for inflammation, SOCS-5 was reported in vascular smooth muscle cells.[14] However, nasal fibroblasts did not increase SOCS-5 signaling by IL-19. Consequently, the signal pathway of IL-19 is inferred to be dependent upon the cell type.

Actually, IL-19 exerts its effect via the type I IL-20 receptor complex consisting of IL-20R1 and IL-20R2. The IL-19 first binds to IL-20R2,[18,19] which leads us to knockdown of IL-20R2 by RNAi in nasal fibroblasts. Recently, Wahl *et al.* reported that IL-20R2 knockout mice were sensitive to the contact allergen. The IL-20 R2 signaling directly regulated CD4 and CD8 T cell response *in vitro* and *in vivo*.[28] Our study showed that regulation of IL-20R2 using the RNAi method abolished the effect of IL-19 on IL-4-induced eotaxin production. These facts suggest that IL-20R2 might be critical for the function of IL-19.

A survey of the distribution of IL-19 protein was performed using healthy human tissues of 28 types with tissue microarray and immunohistochemical staining. The major cell types that stained positive for IL-19 were epithelial cells, endothelial cells, and macrophages.[29] Results showed IL-19 expression to a great degree in airway epithelial cells from asthmatic patients, synergistically activated by IL-13 and IL-17A.[15] We also found IL-19 expression in nasal epithelial cells and infiltrated cells from AR patients. Proinflammatory cytokine IL-1β induced a great increase of IL-19 expression (more than 1000-fold increase) in keratinocytes.[30] The inflammatory response that occurs during cardiopulmonary bypass has often been described as a systemic inflammatory response syndrome resembling sepsis. The level of IL-19 in serum was elevated in cardiac surgery with cardiopulmonary bypass and its rise occurred concomitantly with the induction of IL-10, IL-6, and TNF-α.[31] Endotoxic shock is a systemic inflammatory response to severe bacterial infections. Serum levels of IL-19 are reportedly higher in patients with endotoxic shock than in healthy volunteers.[32] Uremic patients on hemodialysis are in a chronic state of inflammation. Expression of IL-19 correlated with proinflammatory cytokines and Th2 cytokine production in uremic patients on hemodialysis.[33] Psoriasis is a chronic inflammatory skin disease, and in fact, IL-19 expression *in vivo* is elevated in psoriatic skin.[34] In the diseased skin of atopic dermatitis, IL-19 is also highly expressed.[30] These results suggest that IL-19 and several proinflammatory cytokines contribute interactively to inflammatory responses. However, the valuable function of IL-19 remains unclear.

Apoptosis is important as a mechanism of inflammation. However, the regulating mechanism of IL-19 on inflammation has remained controversial. Liao *et al.* demonstrated that treatment of mouse monocyte with IL-19 induced production of IL-6 and TNF-α. Actually, IL-19 also induced mouse monocyte apoptosis and the production of reactive oxygen species.[35] Compared to normal fibroblasts, no difference of trypan blue or PI staining was found in nasal fibroblasts by pretreatment with IL-19 (data not shown). These results suggest that apoptosis is not induced by IL-19 treatment in human nasal fibroblasts.

Anti-proliferative effects of IL-19 on a cancer cell line have been reported.[19] Tian *et al.* also demonstrated a dose-dependent, anti-proliferative effect of IL-19 on primary human coronary artery vascular smooth muscle cells (VSMCs).[14] In contrast, it has been reported that IL-19 induced proliferation of oral cancer cell lines.[29] Results of the present study showed no significant difference of absorbance values by MTT assay evaluating cell growth in nasal fibroblast between pretreatment with or without IL-19 (data not shown). This result demonstrated that the reduction of eotaxin production by pretreatment with IL-19 was not associated with cell growth by nasal fibroblasts.

Fibroblasts established from rheumatoid synovium produce IL-19 and constitutively express both IL-20R1 and IL-20R2. Furthermore, IL-19 induced STAT3 activation and increased IL-6 production by rheumatoid synovium; IL-19 significantly reduced apoptosis of rheumatoid synovium induced by serum starvation.[36] However, nasal fibroblasts were not confirmed by RT-PCR to express IL-19 mRNA (data not shown). Consequently, nasal fibroblasts differ from the fibroblasts of rheumatoid synovium.

Nasal mucosa play a crucial role as the first line of host defense mechanism against invading pathogens. The attack of invading pathogens induces toll-like receptor (TLR) signals in nasal mucosa. Nasal fibroblast and epithelial cells express TLR1, 2, 3, 4, 5, 6, and 9.[13] The TLR signals induce proinflammatory cytokine and expand an allergic inflammation with Th2 cytokine. Viral and bacterial infections clinically produce nasal symptoms to a worse degree in patients with AR. In actuality, SOCS-1 plays an important role in quenching the activation of TLR signal-induced inflammation.[37] Our data imply to us that proinflammatory cytokine induces IL-19 from epithelial cells and that IL-19 affects nasal fibroblasts and suppresses allergic inflammation in an anti-inflammatory role. The regulatory cytokine IL-19 functions in an extremely complicated manner. Our data constitute valuable information that will support additional investigations of the biological function and clinical implications of IL-19 in humans.

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CONFLICT OF INTEREST

No potential conflict of interest was disclosed.

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特集II 好酸球性副鼻腔炎の病態と治療

好酸球性副鼻腔炎の概説*

春名眞一**

Key Words：eosinophilic sinusitis, steroid, endoscopic sinus surgery, asthma, respiratory hypersensitivity

はじめに

近年の慢性副鼻腔炎治療の大きなポイントは，内視鏡手術，マクロライド治療と下気道と関連した副鼻腔炎を示すone airway one diseasesであろう．内視鏡手術は，1980年頃より本格的に慢性副鼻腔炎に導入され，従来の裸眼下手術に比べ格段に繊細で安全な手術操作が可能になった．当然，術後の成績の改善も認められた[1]．

薬物療法としてマクロライド療法が脚光を浴び，14員環マクロライド系抗菌薬の少量長期療法が工藤[2]によってびまん性細気管支炎に著効したと報告された．洲崎(1990年)は慢性副鼻腔炎にも同様な効果があることを報告した[3]．以来，マクロライド療法の基礎的，臨床的研究がなされ，本邦を中心に多くの論文が発表された[4)~7]．欧米においてdouble blind, randomizeでマクロライド療法の有用性が証明されている[8]．さらに，内視鏡手術後にマクロライド療法は使用しない場合に比べ有意に改善効果が高いことも示された[9]．

一方，マクロライド療法の無効例が選別できるようになった．マクロライド療法のガイドライン[10]によると，①I型アレルギー性炎症が主体である症例，②気管支喘息を合併している症例，③中鼻道が高度に閉塞している症例，④大きな鼻茸を有する症例，⑤長期投与中に急性増悪を生じた症例としている．Harunaらは，鼻腔ポリープを切除前後におけるマクロライド療法の効果を比較すると，多発性ポリープの切除後のマクロライド療法で有意に改善したと報告している[11]．さらに，採取したポリープ中の好酸球浸潤が高くなるほど，マクロライド療法の効果が不良であるとされた．特に喘息合併例では，非合併例に比べ有意に不良を示した．喘息合併した副鼻腔炎は，従来，難治性副鼻腔炎と認識されてきた[12]．

好酸球性副鼻腔炎の定義

好酸球性副鼻腔炎は，欧米においても同様な好酸球性副鼻腔炎についての報告があり，Diffuse Eosinophil-dominated polyposis[21]やEosinophilic Mucin Rhinosinusitis[22]とされる．しかしながら，明確な診断基準はない[23]． 春名らは，2001年に副鼻腔粘膜に著名な好酸球浸潤をきたした副鼻腔炎を好酸球性副鼻腔炎と提唱した[13]．好酸球性幅鼻腔炎は従来の慢性化膿性副鼻腔炎と異なる多くの臨床的特徴を有する．診断(案)を図1に示す．絶対条件として成人発症，両側性副鼻腔炎であること，CT画像で篩骨洞陰影が上顎洞に比べて優位であること，自覚症状として嗅覚障害が出現しやすいことがあげられる[14]．さらに，

* The conception of eosinophilic sinusitis.

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絶対条件

- 成人発症*1
- 両側性副鼻腔病変
- CT所見で上顎洞より篩骨洞の陰影が優位
- 主訴の中に嗅覚障害がある
- 内視鏡鼻内所見で上中鼻道，上中鼻甲介に鼻ポリープを認める*2
- 血中好酸球数6％(300個/ml)以上あるいは副鼻腔組織中好酸球数100個以上*3で好酸球優位

付帯条件

- ステロイド薬，特に経口ステロイド薬が臨床所見の改善に有効
- 気管支喘息，アスピリン喘息を合併する*4
- 内視鏡下鼻内副鼻腔手術後に経過不良を呈する*5
- マクロライド療法の効果は不明
- 粘稠性分泌物が認められる

*1　稀に10歳代に認める
*2　鼻ポリープを観察できにくい症例もある
*3　HE染色，400倍視野で好酸球数の多く存在する3か所で計測した平均値
　　ただし，血中および組織中好酸球数は病状および薬物により変動する可能性がある
*4　喘息を合併しないこともある
*5　術後1年で篩骨洞内視鏡所見を良好，やや良好，不良にわけると1/4で不良例を呈する

図1　好酸球性副鼻腔炎のCriteria(案)

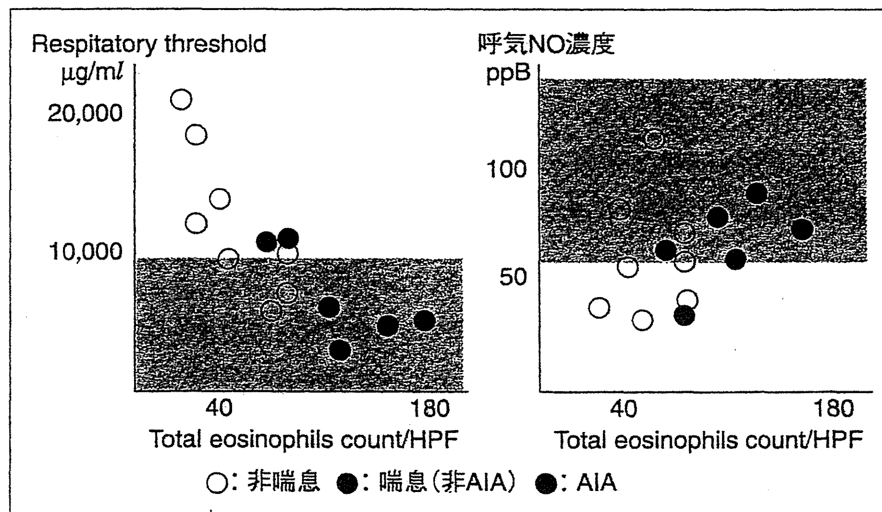

図2　高度副鼻腔炎における下気道過敏性と呼気NO濃度

血中好酸球6％(300個/μl)以上あるいは副鼻腔組織中好酸球数100個以上で好酸球優位とされる．10歳代では好酸球性副鼻腔炎を認めることはきわめて稀であり，筆者自身も今まで2例のみ経験した．喘息でのnonatopic typeの特徴と類似している．気管支喘息を合併する場合も多く，従来は喘息合併副鼻腔炎と呼ばれ，難治性副鼻腔炎の代名詞とされた．しかし，必ずしも喘息を合併しない場合でも上記の診断基準に合致する場合は多い．図2のように，高度副鼻腔炎症例に下気道過敏性試験や呼気中NO濃度を検査すると，喘息が合併しなくとも有意に過敏性が更新している症例が存在する．図3の症例のように，術前に好酸球性副鼻腔炎との認識がなく，術後長期間の観察なしで放置され，数年後に経過不良となってしまうこともある．上気道では，好酸球増多を示し，典型的な好酸球性副鼻腔炎を示し，将来，喘息の発症する可能性があるのか

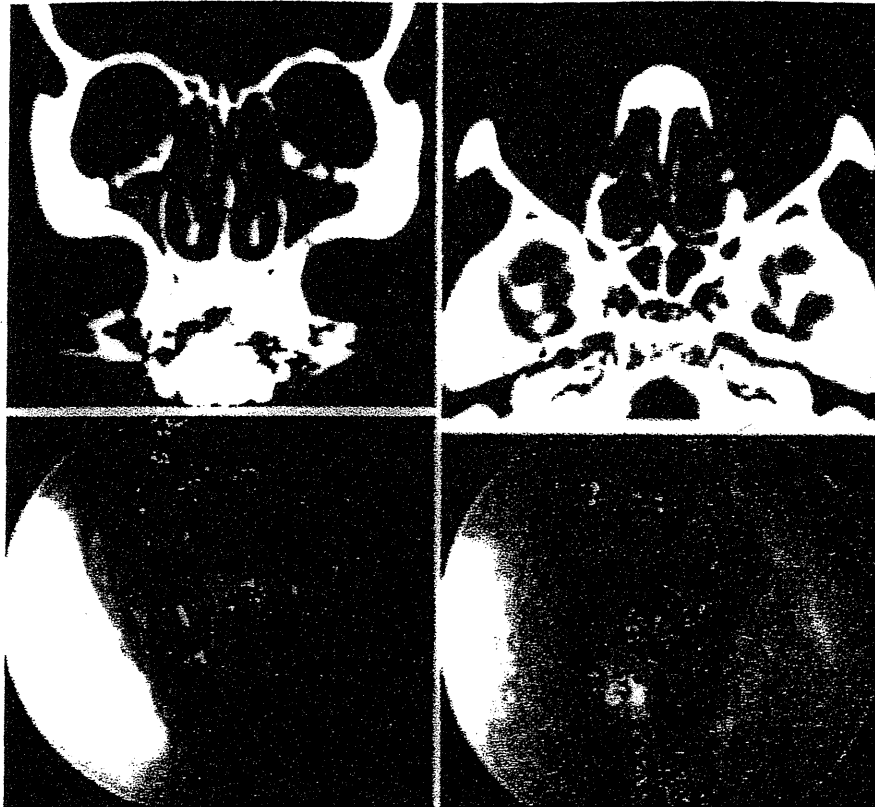

図3　喘息合併なしの好酸球性副鼻腔炎
CTで汎副鼻腔炎(上段)と鼻内内視鏡所見で多発性ポリープがある(下段)．嗅覚障害≧鼻閉，血中好酸球10.2%，血清総IgE値低値，RAST(−)，家族歴：息子が喘息，過敏性：2,500μg/ml，eNO：484ppB，術後2年で再燃し，鼻閉，鼻漏改善，嗅覚障害あり，血中好酸球数7.2%，プレドニン10mg 2週間，5mg 1週間投与し，その後に自宅鼻洗浄，抗LT薬，リノコート点鼻に改善した．

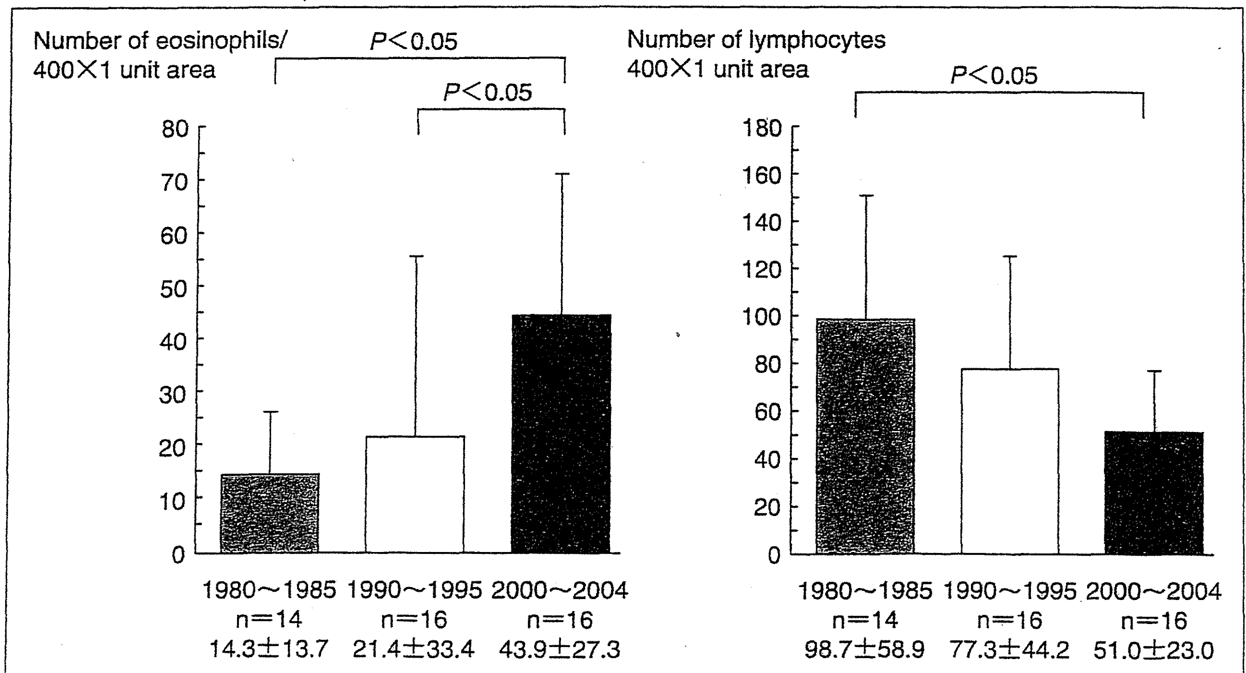

図4　年代別，組織中の好酸球数，好中球の推移
鼻ポリープ中の好酸球数の増加と逆に好中球数の減少が認められる．(文献[15]より引用)

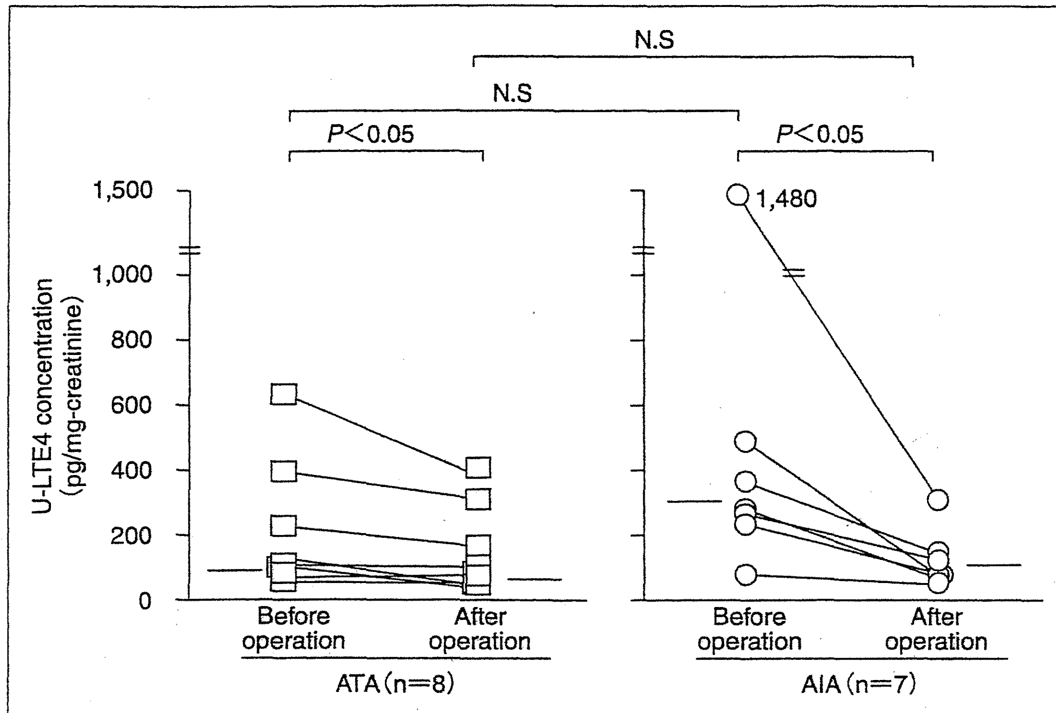

図5　副鼻腔炎を伴う喘息での鼻手術前後の尿中LT濃度の比較
喘息合併やアスピリン喘息合併副鼻腔炎の鼻手術前後で有意に尿中LT濃度が減少した．
（文献[18]より引用）

もしれない．洲崎の報告では1980～1985年，1990～1995年，2000～2004年での鼻ポリープ中の好酸球数と好中球数を比較すると有意に好酸球の増加と好中球の減少を示している[15]（図4）．好酸球数と下気道過敏性は相関すると報告されており，上気道の好酸球増多は，下気道に悪影響を及ぼしていると考えられる．石戸谷も診断基準を提唱しており，症状（早期からの嗅覚障害，鼻閉，粘稠な鼻汁），鼻内所見（両側性，多発性鼻茸または中鼻甲介周囲の粘膜浮腫），CT画像（篩骨洞優位な陰影，嗅裂の閉塞，副鼻腔内に高輝度の分泌物），血液検査（血中好酸球数の増多（≧6％または≧400個/μl）の4項目をあげている[16]．

好酸球性副鼻腔炎の病態

最近では，上気道炎症，特に鼻副鼻腔炎と下気道炎症とを関連した病態を考えることが多い（one airway one disease）．従来よりSinobronchitisという言葉があり，慢性中耳炎—慢性化膿性副鼻腔炎—慢性気管支炎という一連の流れはあった．そして，副鼻腔炎治療を徹底することで下気道病変を改善させることができるとされた．もう一つは，好酸球性中耳炎—好酸球性副鼻腔炎—気管支喘息（アスピリン喘息）である．また，上記の副鼻腔炎を好中球優位（IL-5 positive nasal polyps）と好酸球優位（IL-5 negative nasal polyps）とも提唱されている[17]．気管支喘息やアスピリン喘息で尿中LTE4濃度を計測すると，副鼻腔炎なしに比べて副鼻腔炎を合併した症例では有意に高い濃度が認められ，かつ鼻内視鏡手術をすることで術前に比べ優位に減少するとされる[18]（図5）．したがって，上気道から多量のLTが産生され，過剰なLT産生が下気道になんらかの悪影響を与えていると考えられる．鼻ポリープ中のロイコトリエン濃度を喘息合併群，アスピリン喘息合併群，鼻アレルギー合併群とコントロール群と比較するとCysLT濃度は有意に喘息合併群，アスピリン喘息合併群で高く，逆にPGE2は有意に低いとされ[19]，上気道においてもアラキドン酸代謝でロイコトリエンへ過剰産生されていることが考えられる[20]．