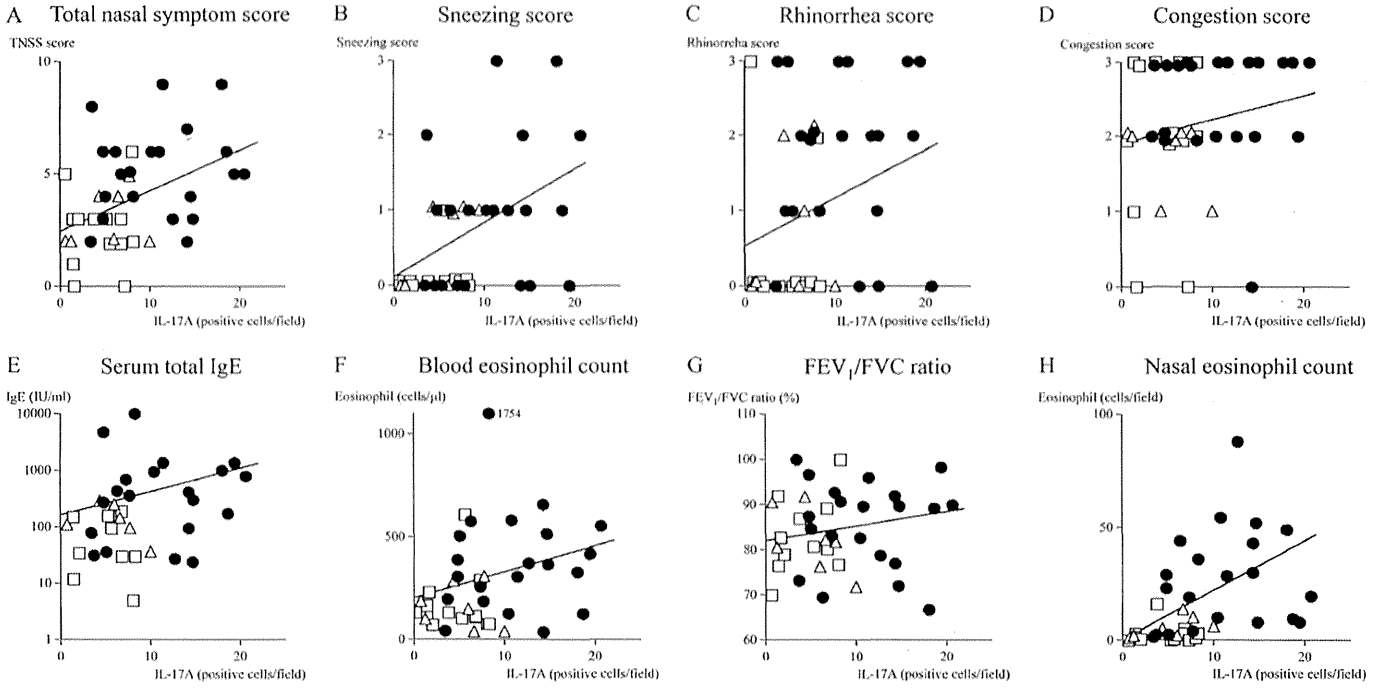

Figure 3. Relationship between numbers of interleukin (IL)-17A$^+$ cells in nasal mucosa and pathophysiological characterizations including (A) total nasal symptom score (TNSS), (B) sneezing score, (C) rhinorrhea score, (D) congestion score, (E) serum total IgE levels, (F) blood eosinophil count, (G) 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.

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MAIN ARTICLE

Pulmonary function in patients with chronic rhinosinusitis and allergic rhinitis

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Abstract

Background: A close relationship between upper and lower respiratory tract diseases has been reported. However, little is known about pulmonary function in patients with upper respiratory tract diseases.

Methods: Pulmonary function was measured in: 68 patients with chronic rhinosinusitis without nasal polyps, 135 patients with chronic rhinosinusitis with nasal polyps, 89 patients with allergic rhinitis and 100 normal control subjects. The relationships between pulmonary function and clinical parameters were assessed. These parameters included radiographic severity of chronic rhinosinusitis, serum total immunoglobulin E levels, concentrations of cytokines in nasal secretions and exhaled nitric oxide levels.

Results: The pulmonary function of patients with chronic rhinosinusitis was significantly affected. The level of interleukin-5 in nasal secretions was significantly correlated with pulmonary function in patients with chronic rhinosinusitis.

Conclusion: The findings indicated latent obstructive lung function changes in chronic rhinosinusitis patients. The cytokines in nasal secretions might be related to obstructive lung function changes in chronic rhinosinusitis.

Key words: Sinusitis; Rhinitis; Asthma; Chronic Obstructive Pulmonary Disease; COPD; Lung Function Tests

Introduction

Chronic rhinosinusitis is defined as a persistent inflammatory response involving the mucous membranes of the nasal cavity and paranasal sinuses. It has recently been divided into two subgroups: chronic rhinosinusitis with nasal polyps, and chronic rhinosinusitis without nasal polyps.[1] Allergic rhinitis is characterised by a number of symptoms, including sneezing, nasal congestion, nasal itching and rhinorrhoea.[2] Chronic rhinosinusitis and allergic rhinitis are common upper respiratory tract diseases.[3–5] The presence of allergic rhinitis is one of the risk factors for the development of asthma; the association between allergic rhinitis and asthma is explained by the 'united airway disease' hypothesis.[2,6] It has been suggested that chronic obstructive pulmonary disease (COPD) is also associated with upper airway diseases including chronic rhinosinusitis.[7–9]

Although numerous studies have described a relationship between upper and lower respiratory tract diseases, pulmonary function in patients with upper respiratory tract diseases has not been fully examined. To the best of our knowledge, no study has compared pulmonary function in patients with upper respiratory tract diseases (chronic rhinosinusitis and allergic rhinitis) with that in normal controls. This study aimed to evaluate pulmonary function in patients with chronic rhinosinusitis or allergic rhinitis who had not been diagnosed with lower respiratory tract diseases.

Materials and methods

This study was approved by the Institutional Review Board of Okayama University (approval number, RINRI-877), and was conducted in compliance with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all enrolled subjects.

Subjects

Four groups of participants were enrolled in this study: a chronic rhinosinusitis without nasal polyps group, a chronic rhinosinusitis with nasal polyps group, an allergic rhinitis group and a normal control group.

A total of 203 chronic rhinosinusitis patients who were scheduled to undergo functional endoscopic sinus surgery (FESS) at Okayama University were recruited and divided into two groups (chronic rhinosinusitis without nasal polyps and chronic rhinosinusitis with nasal polyps groups). The diagnosis of chronic

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rhinosinusitis with nasal polyps was based on the definition in the European Position Paper on Rhinosinusitis and Nasal Polyps 2012.[1] All chronic rhinosinusitis patients were resistant to medical treatment, including macrolide therapy.[10] Chronic rhinosinusitis patients with chronic lower lung diseases including bronchial asthma and COPD were excluded from this study. The diagnoses of asthma and COPD were based on the internationally accepted clinical guidelines.[11,12]

Eighty-nine patients with allergic rhinitis took part in this study. Allergic rhinitis was defined according to the clinical symptoms and serological results reported in the *Practical Guideline for the Management of Allergic Rhinitis in Japan* (2008).[13] The radioallergosorbent test was used for the diagnosis of immunoglobulin E (IgE) mediated allergic reactions. Computed tomography (CT) was performed to exclude the possibility of coexisting paranasal sinus abnormalities. Allergic rhinitis patients who were clinically diagnosed as having lower respiratory tract diseases were excluded from this study.

Age-matched, normal control subjects with no chronic respiratory diseases were also recruited ($n = 100$).

Because cigarette smoking could affect pulmonary function, smoking status was examined and the Brinkman Index (number of cigarettes per day × smoking years) was calculated.

Pulmonary function tests

Prior to FESS, pulmonary function testing was performed with the Chestac-9800 spirometer (Chest MI, Tokyo, Japan) according to the standardisation of lung function tests of the American Thoracic Society and European Respiratory Society.[14] The following parameters were measured or calculated: percentage of predicted vital capacity; forced expiratory volume in 1 second; percentage of predicted forced expiratory volume in 1 second; forced expiratory volume in 1 second / forced vital capacity ratio; mean forced expiratory flow between 25 and 75 per cent of the forced vital capacity; peak expiratory flow; maximal expiratory flow rate at 50 per cent of vital capacity; maximal expiratory flow rate at 25 per cent of vital capacity; and the maximal expiratory flow rate at 50 per cent of vital capacity / maximal expiratory flow rate at 25 per cent of vital capacity ratio.

Rhinomanometry

In all chronic rhinosinusitis patients, nasal obstruction was examined (prior to FESS) by active anterior rhinomanometry with a nasal nozzle at air pressure 100 Pa (MPR-3100; Nihon Kohden, Tokyo, Japan), according to the manufacturer's instructions.[15]

Chronic rhinosinusitis assessment

The radiographic severity of chronic rhinosinusitis was assessed (prior to FESS) using the Lund–MacKay CT staging system.[16]

Blood tests

Blood samples were taken prior to FESS. The peripheral blood eosinophil count was determined. Serum total IgE levels were measured with the ImmunoCap 250 system (Phadia AB, Uppsala, Sweden), according to the manufacturer's protocols.

Inflammatory mediators assessment

Nasal secretion was collected (prior to FESS) from 13 randomly selected chronic rhinosinusitis patients without lung disease (mean age ± standard deviation (SD), 48.2 ± 12.5 years; i.e. 3 chronic rhinosinusitis patients without nasal polyps and 10 chronic rhinosinusitis patients with nasal polyps). A bicinchoninic acid assay was performed to quantify the total protein concentration in each sample using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Rockford, Illinois, USA). The concentrations of inflammatory mediators (tumour necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-4, IL-5, IL-6, IL-8, IL-17 and interferon-γ) were determined by BD OptEIA enzyme-linked immunosorbent assay sets (BD, Franklin Lakes, New Jersey, USA). A zero value was assigned when the concentration of inflammatory mediators was under the detection limit of the enzyme-linked immunosorbent assay set. The concentrations of TNF-α, IL-1β, IL-4, IL-5, IL-6, IL-8, IL-17 and interferon-γ (pg/ml) were divided by the concentration of total protein of each sample (mg of total protein per ml) for standardisation. The calculated concentrations of each cytokine (pg/mg total protein) were used for statistical evaluation.

Exhaled nitric oxide concentration

The Niox Mino device (Aerocrine AB, Solna, Sweden) was used to measure the level (fraction) of exhaled nitric oxide according to the manufacturer's instructions. This was carried out (prior to FESS) in 13 randomly selected chronic rhinosinusitis patients without lung disease (mean age ± SD, 48.2 ± 12.5 years; i.e. 3 chronic rhinosinusitis patients without nasal polyps and 10 chronic rhinosinusitis patients with nasal polyps).

Statistical analysis

Values are presented as means ± SD. Differences in proportions were examined using the chi-square test. For comparisons between groups, a one-way analysis of variance was conducted to establish the significance of inter-group variability. The two-tailed unpaired *t*-test was then used for between-group comparisons for normally distributed data. A correlation analysis was performed using Spearman's rank correlation coefficient. *P* values less than 0.05 were considered significant. Statistical analyses were performed with the Statistical Package for the Social Sciences software (SPSS, Chicago, Illinois, USA).

Results

Subject characteristics

Demographic data are presented in Table I. There was a significantly higher ratio of males to females in the chronic rhinosinusitis group compared with the normal control group. There were no significant differences in age or smoking status among the groups.

Pulmonary function

Pulmonary function data for patients with chronic rhinosinusitis (without any clinically diagnosed lung disease) and normal control subjects are shown in Figure 1. There were no significant differences between chronic rhinosinusitis patients and normal controls in terms of forced expiratory volume in 1 second and the percentage of predicted vital capacity. However, pulmonary function was significantly affected in chronic rhinosinusitis patients (compared with normal controls) in the following parameters: percentage of predicted forced expiratory volume in 1 second; forced expiratory volume in 1 second / forced vital capacity ratio; peak expiratory flow; mean forced expiratory flow between 25 and 75 per cent of the forced vital capacity; maximal expiratory flow rate at 50 per cent of vital capacity; maximal expiratory flow rate at 25 per cent of vital capacity; and maximal expiratory flow rate at 50 per cent of vital capacity / maximal expiratory flow rate at 25 per cent of vital capacity ratio. No significant differences were observed between the chronic rhinosinusitis without nasal polyps group and the chronic rhinosinusitis with nasal polyps group in any parameters.

In patients with allergic rhinitis, the percentage of predicted vital capacity was 114.9 ± 15.8 per cent, the forced expiratory volume in 1 second was 3.58 ± 0.75 litres per second, the percentage of predicted forced expiratory volume in 1 second was 106.0 ± 11.8 per cent, the forced expiratory volume in 1 second / forced vital capacity ratio was 84.2 ± 7.73 per cent, the peak expiratory flow was 8.76 ± 1.98 litres per second, the mean forced expiratory flow between 25 and 75 per cent of the forced vital capacity was 3.56 ± 1.20 litres per second, the maximal expiratory flow rate at 50 per cent of vital capacity was 4.21 ± 1.24 litres per second, the maximal expiratory flow rate at 25 per cent of vital capacity was 1.63 ± 0.82 litres per second, and the maximal expiratory flow rate at 50 per cent of vital capacity / maximal expiratory flow rate at 25 per cent of vital capacity ratio was 3.10 ± 1.76. No significant differences in pulmonary function parameters were seen between allergic rhinitis patients and normal controls.

Nasal obstruction

The factors that might affect pulmonary function in chronic rhinosinusitis patients were investigated. Rhinomanometry was used to evaluate nasal obstruction. The mean nasal resistances at delta P (transnasal differential pressure) 100 Pa in the chronic rhinosinusitis without nasal polyps group was 0.32 ± 0.23 Pa/cm^3/s, and in the chronic rhinosinusitis with nasal polyps group it was 0.34 ± 0.24 Pa/cm^3/s. There was no significant difference in nasal resistance between the chronic rhinosinusitis groups ($p = 0.772$). No significant correlations were observed between nasal resistance and pulmonary function in either of the chronic rhinosinusitis groups (Tables II and III).

Computed tomography score

The Lund–Mackay CT score was used to evaluate chronic rhinosinusitis severity. The average Lund–Mackay scores on pre-operative CT scans were 6.75 ± 4.40 in the chronic rhinosinusitis without nasal polyps group and 11.71 ± 5.75 in the chronic rhinosinusitis with nasal polyps group; this difference was significant ($p < 0.001$). No significant correlations were observed between pre-operative CT score and pulmonary function in either of the chronic rhinosinusitis groups (Tables II and III).

Peripheral blood eosinophil count

The mean peripheral blood eosinophil count was 204.9 ± 162.8 in the chronic rhinosinusitis without

TABLE I
SUBJECT CHARACTERISTICS

Parameter	CRSsNP	CRSwNP	AR	Normal	*p*
Subjects (*n*)	68	135	89	100	
Male/female (*n*)	41/27	91/44	64/25	51/49	0.014*
Age (years)	39.5 ± 11.4	37.4 ± 11.9	37.1 ± 14.1	38.7 ± 10.8	0.531†
Smoking status					
– Ex	12/68 (17.6%)	26/135 (19.3%)	20/89 (22.5%)	25/100 (25.0%)	0.678*
– Current	20/68 (29.4%)	40/135 (29.6%)	22/89 (24.7%)	20/100 (20.0%)	
– Never	36/68 (52.9%)	69/135 (51.1%)	47/89 (52.8%)	55/100 (55.0%)	
Brinkman index					
– All smokers	438.2 ± 309.3	383.6 ± 305.3	335.7 ± 369.9	313.2 ± 289.3	0.328†
– Ex-smokers	380.2 ± 323.5	360.0 ± 291.1	335.5 ± 442.2	307.0 ± 301.5	0.919†
– Current smokers	473.0 ± 303.4	399.0 ± 316.9	335.9 ± 300.5	321.0 ± 280.9	0.364†

Data represent means ± standard deviation unless specified otherwise. *Chi-square test. †One-way analysis of variance. CRSsNP = chronic rhinosinusitis without nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps; AR = allergic rhinitis

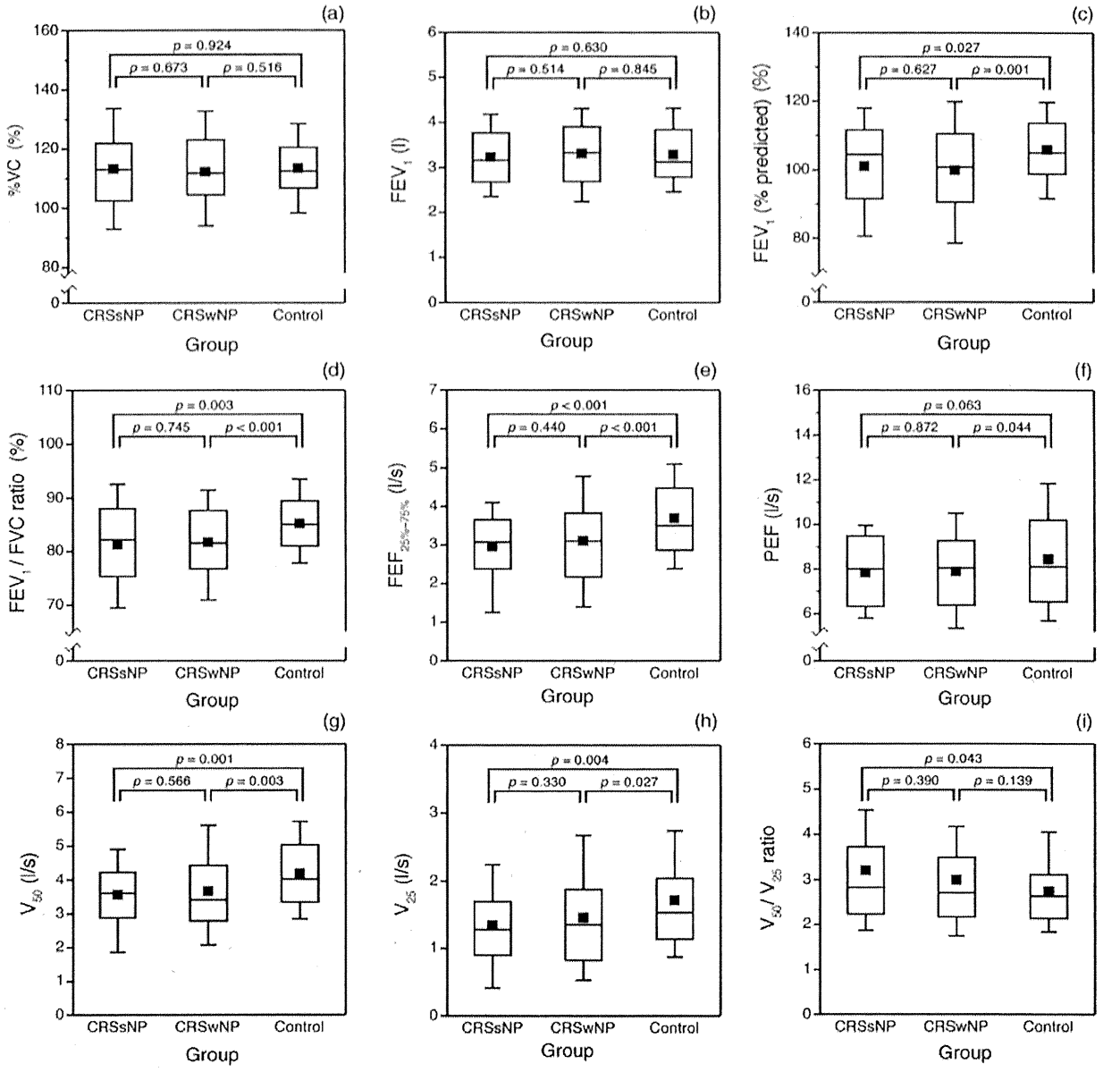

FIG. 1

Pulmonary function in patients with chronic rhinosinusitis, specifically: (a) percentage of predicted vital capacity (%VC); (b) forced expiratory volume in 1 second (FEV_1); (c) percentage of predicted forced expiratory volume in 1 second (FEV_1); (d) forced expiratory volume in 1 second / forced vital capacity (FEV_1/FVC) ratio; (e) mean forced expiratory flow between 25 and 75 per cent of the forced vital capacity ($FEF_{25\%-75\%}$); (f) peak expiratory flow (PEF); (g) maximal expiratory flow rate at 50 per cent of vital capacity (V_{50}); (h) maximal expiratory flow rate at 25 per cent of vital capacity (V_{25}); and (i) maximal expiratory flow rate at 50 per cent of vital capacity / maximal expiratory flow rate at 25 per cent of vital capacity (V_{50}/V_{25}) ratio. (Rectangles include range from 25th to 75th percentile, horizontal lines indicate median, vertical lines indicate range from 10th to 90th percentile and black squares represent mean value.) CRSsNP = chronic rhinosinusitis without nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps

nasal polyps group and 343.6 ± 311.4 in the chronic rhinosinusitis with nasal polyps group; this difference was significant ($p < 0.001$). There was no significant correlation between peripheral blood eosinophil count and pulmonary function for either chronic rhinosinusitis group (Tables II and III).

Immunoglobulin E level

The mean total serum IgE level was 344.0 ± 494.7 IU/ml in the chronic rhinosinusitis without nasal polyps group and 268.6 ± 455.8 IU/ml in the chronic rhinosinusitis with nasal polyps group; this difference was not significant. There was no significant correlation between serum IgE level and pulmonary function for either chronic rhinosinusitis group (Tables II and III).

Inflammatory mediators

The mean concentrations of tumour necrosis factor-α, interleukin (IL)-1β, IL-4, IL-5, IL-6, IL-8 and interferon-γ in nasal secretions were 3.4 ± 0.6, 14.1 ± 17.7, 6.4 ± 6.3, 3.5 ± 2.2, 3.9 ± 1.9, 112.4 ± 43.3 and 2.6 ± 1.0 pg/mg total protein, respectively. Interleukin-17 was undetectable in all samples. The level of IL-5 was significantly correlated with

TABLE II
PULMONARY FUNCTION AND CLINICAL PARAMETERS CORRELATION: PATIENTS WITHOUT NASAL POLYPS

Parameter	Nasal resistance		CT score		Blood eosinophil count		Serum IgE level	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
%VC	0.276	0.339	−0.195	0.123	−0.145	0.251	−0.126	0.420
FEV_1	0.057	0.841	−0.216	0.087	−0.019	0.876	−0.004	0.978
$\%FEV_1$	0.287	0.299	−0.290	0.020	−0.228	0.065	−0.058	0.708
FEV_1:FVC	0.135	0.632	−0.121	0.342	−0.087	0.490	−0.007	0.966
PEF	−0.162	0.565	−0.169	0.180	0.097	0.441	0.237	0.122
$FEF_{25\%-75\%}$	0.089	0.754	−0.108	0.397	−0.027	0.827	−0.005	0.975
V_{50}	−0.011	0.969	−0.167	0.187	−0.108	0.387	−0.033	0.830
V_{25}	0.188	0.502	0.014	0.911	0.068	0.585	−0.048	0.756
V_{50}:V_{25}	−0.303	0.272	0.139	0.274	0.161	0.197	0.039	0.800

CT = computed tomography; IgE = immunoglobulin E; %VC = percentage of predicted vital capacity; FEV_1 = forced expiratory volume in 1 second; $\%FEV_1$ = percentage of predicted forced expiratory volume in 1 second; FEV_1:FVC = forced expiratory volume in 1 second / forced vital capacity ratio; PEF = peak expiratory flow; $FEF_{25\%-75\%}$ = mean forced expiratory flow between 25 and 75 per cent of forced vital capacity; V_{50} = maximal expiratory flow rate at 50 per cent of vital capacity; V_{25} = maximal expiratory flow rate at 25 per cent of vital capacity; V_{50}:V_{25} = maximal expiratory flow rate at 50 per cent of vital capacity / maximal expiratory flow rate at 25 per cent of vital capacity ratio

pulmonary function (forced expiratory volume in 1 second / forced vital capacity ratio, $p = 0.048$; mean forced expiratory flow between 25 and 75 per cent of the forced vital capacity, $p = 0.027$; maximal expiratory flow rate at 50 per cent of vital capacity, $p = 0.043$; maximal expiratory flow rate at 25 per cent of vital capacity, $p = 0.043$; maximal expiratory flow rate at 50 per cent of vital capacity / maximal expiratory flow rate at 25 per cent of vital capacity ratio, $p = 0.032$) (Table IV).

Exhaled nitric oxide

The mean level of exhaled nitric oxide was 27.8 ± 17.1 parts per billion. There were no significant correlations between levels of exhaled nitric oxide and each pulmonary function test result.

Discussion

Recent studies have shown a strong link between asthma and allergic rhinitis, and COPD may also be associated with upper airway involvement.[2,9,17–19] Patients with asthma and COPD show increased nasal symptoms and more nasal inflammation.[7]

Although numerous studies have reported an association between upper and lower airway diseases based on the concept of the 'united airway disease' hypothesis, pulmonary function in patients with upper airway diseases has not been fully examined.[20] One study reported spirometric abnormalities in patients with allergic rhinitis, but there was no normal control group in that study.[21] Furthermore, no previous study has investigated pulmonary function in chronic rhinosinusitis patients without lower respiratory tract disease. The present study showed, for the first time, that patients with chronic rhinosinusitis had latent obstruction of the small airway.

The effects of allergic rhinitis and chronic rhinosinusitis on lung function in patients with lower lung disease remain controversial. A recent report noted that

TABLE III
PULMONARY FUNCTION AND CLINICAL PARAMETERS CORRELATION: PATIENTS WITH NASAL POLYPS

Parameter	Nasal resistance		CT score		Blood eosinophil count		Serum IgE level	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
%VC	0.050	0.681	−0.036	0.689	−0.012	0.889	−0.105	0.262
FEV_1	−0.191	0.109	0.029	0.739	−0.024	0.787	0.055	0.551
$\%FEV_1$	0.039	0.749	0.021	0.813	−0.104	0.241	−0.158	0.086
FEV_1:FVC	−0.083	0.491	−0.084	0.342	−0.075	0.395	0.020	0.832
PEF	−0.090	0.457	−0.028	0.755	−0.068	0.444	0.009	0.926
$FEF_{25\%-75\%}$	−0.141	0.239	−0.034	0.699	−0.075	0.397	0.031	0.742
V_{50}	−0.111	0.357	−0.044	0.620	−0.047	0.599	0.001	0.988
V_{25}	−0.140	0.243	−0.028	0.754	−0.104	0.241	0.050	0.590
V_{50}:V_{25}	−0.018	0.879	0.018	0.842	0.207	0.018	0.017	0.857

CT = computed tomography; IgE = immunoglobulin E; %VC = percentage of predicted vital capacity; FEV_1 = forced expiratory volume in 1 second; $\%FEV_1$ = percentage of predicted forced expiratory volume in 1 second; FEV_1:FVC = forced expiratory volume in 1 second / forced vital capacity ratio; PEF = peak expiratory flow; $FEF_{25\%-75\%}$ = mean forced expiratory flow between 25 and 75 per cent of forced vital capacity; V_{50} = maximal expiratory flow rate at 50 per cent of vital capacity; V_{25} = maximal expiratory flow rate at 25 per cent of vital capacity; V_{50}:V_{25} = maximal expiratory flow rate at 50 per cent of vital capacity / maximal expiratory flow rate at 25 per cent of vital capacity ratio

TABLE IV
PULMONARY FUNCTION AND NASAL SECRETION CYTOKINE LEVELS CORRELATION

Parameter	TNF-α		IL-1β		IL-4		IL-5		IL-6		IL-8		IFN-γ	
	r	*p*	*r*	*p*	*r*	*p*	*R*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
%VC	0.467	0.108	−0.111	0.718	−0.103	0.737	−0.258	0.394	0.120	0.696	0.369	0.214	−0.490	0.089
FEV_1	0.312	0.299	−0.373	0.209	−0.377	0.204	−0.531	0.062	−0.162	0.597	−0.031	0.919	−0.470	0.105
$\%FEV_1$	0.448	0.124	−0.018	0.953	−0.030	0.921	−0.037	0.904	0.004	0.988	0.236	0.437	−0.147	0.632
FEV_1:FVC	−0.177	0.562	−0.564	0.044	−0.523	0.066	−0.557	0.048	−0.434	0.138	−0.442	0.130	−0.058	0.850
PEF	0.328	0.274	−0.126	0.681	−0.187	0.541	−0.263	0.385	−0.154	0.621	−0.140	0.649	−0.129	0.676
$FEF_{25\%-75\%}$	−0.017	0.955	−0.543	0.055	−0.502	0.080	−0.609	0.027	−0.441	0.131	−0.373	0.209	−0.219	0.471
V_{50}	0.323	0.282	−0.512	0.073	−0.509	0.075	−0.568	0.043	−0.407	0.167	−0.382	0.197	−0.157	0.609
V_{25}	−0.288	0.340	−0.498	0.082	−0.438	0.133	−0.567	0.043	−0.430	0.143	−0.360	0.227	−0.219	0.472
V_{50}:V_{25}	0.647	0.017	0.580	0.037	0.496	0.084	0.594	0.032	0.269	0.375	0.268	0.377	0.347	0.246

TNF = tumour necrosis factor; IL = interleukin; IFN = interferon; %VC = percentage of predicted vital capacity; FEV_1 = forced expiratory volume in 1 second; $\%FEV_1$ = percentage of predicted forced expiratory volume in 1 second; FEV_1:FVC = forced expiratory volume in 1 second / forced vital capacity ratio; PEF = peak expiratory flow; $FEF_{25\%-75\%}$ = mean forced expiratory flow between 25 and 75 per cent of forced vital capacity; V_{50} = maximal expiratory flow rate at 50 per cent of vital capacity; V_{25} = maximal expiratory flow rate at 25 per cent of vital capacity; V_{50}:V_{25} = maximal expiratory flow rate at 50 per cent of vital capacity / maximal expiratory flow rate at 25 per cent of vital capacity ratio

asthmatics without rhinitis tend to have poorer lung function than asthmatic patients with rhinitis.[6,22] In the present study, it was clear that patients with chronic rhinosinusitis had a normal percentage of predicted vital capacity. However, compared with normal control subjects, the following parameters were affected: percentage of predicted forced expiratory volume in 1 second; forced expiratory volume in 1 second / forced vital capacity ratio; peak expiratory flow; mean forced expiratory flow between 25 and 75 per cent of the forced vital capacity; maximal expiratory flow rate at 50 per cent of vital capacity; maximal expiratory flow rate at 25 per cent of vital capacity; and maximal expiratory flow rate at 50 per cent of vital capacity / maximal expiratory flow rate at 25 per cent of vital capacity ratio. These findings suggest that chronic rhinosinusitis patients who are not clinically diagnosed as having lung disease do show evidence of obstructive lung function changes, even if these changes are asymptomatic. In contrast, there were no significant differences between allergic rhinitis patients and control subjects in spirometric parameters.

The present study investigated the factors that might influence obstructive lung function in chronic rhinosinusitis patients. Rhinomanometry is a sensitive and specific technique for the measurement of nasal obstruction.[23] The upper respiratory tract has important roles, including acting as a physical filter, resonator, heat exchanger and humidifier of inhaled air.[24] The conditions leading to nasal obstruction may trigger lower airway dysfunction.[7] The CT score based on the Lund–Mackay staging system is commonly used to assess the extent and severity of inflammatory changes in chronic rhinosinusitis.[1,25] Deal and Kountakis reported that the CT score was greater in chronic rhinosinusitis with nasal polyps patients than in chronic rhinosinusitis without nasal polyps patients.[26] Although the presence of polyps in the nasal area causes blocked nose, nasal resistance to airflow (measured by rhinomanometry and CT score) was not significantly correlated with lung function in the present study.

Peripheral blood eosinophil count and serum IgE level are widely used to evaluate patients with various allergic diseases, including asthma and allergic rhinitis.[27,28] In the present study, no relationship was found between pulmonary function and these inflammatory mediators.

Various explanations for the upper and lower airway association have been presented. These hypotheses include: systemic reactions; nasobronchial reflex; pharyngobronchial reflex; post-nasal drainage of inflammatory mediators from the upper to lower airways; and inhalation of dry, cold air and environmental pollutants.[24,29–31] In an animal study by Kogahara *et al.*, it was evident that a viscous post-nasal drip could flow into the lower respiratory organs when the host was asleep.[32] Cytokines and chemokines are important factors in the pathogenesis of upper respiratory

diseases, and they play a key role in asthma and COPD.[33,34] The present study showed that patients with increased nasal interleukin-5 levels had asymptomatic lung lesions. Although the number of samples was limited, the present study findings suggest that post-nasal drip containing cytokines might be associated with obstructive lung injury in patients with chronic rhinosinusitis.

- **A close relationship has been reported between upper and lower respiratory disease**
- **Spirometry indicated obstructive lung function in chronic rhinosinusitis patients without lower respiratory tract disease**
- **Cytokines in nasal secretions might be related to lung function**

Exhaled nitric oxide is a marker of airway inflammation, and the concentration of exhaled nitric oxide is elevated in patients with bronchial asthma, COPD, and chronic rhinosinusitis with nasal polyps.[35–38] In the present study, no significant correlation was found between exhaled nitric oxide level and pulmonary function test parameters.

Conclusion

Chronic rhinosinusitis patients without clinically diagnosed lung disease had latent lung obstruction. The chronic rhinosinusitis patients with decreased lung function may be in danger of developing lower respiratory disease. Our findings suggest that the patients with upper respiratory disease should be followed carefully in order to detect lung disease. Several factors in the upper respiratory tract are considered as potential explanations for the effects on lung function. Among these factors, the present findings suggest that cytokines in nasal secretions might be related to lung obstruction.

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ORIGINAL ARTICLE

Cellular Responses to *Staphylococcus aureus* Alpha-Toxin in Chronic Rhinosinusitis with Nasal Polyps

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ABSTRACT

Background: In contrast to *Staphylococcus aureus*-derived superantigenic exotoxins, the role of non-superantigenic exotoxins in the pathogenesis of eosinophilic airway diseases remains obscure. We sought to characterize *S. aureus* alpha-toxin-induced cellular responses in chronic rhinosinusitis with nasal polyps (CRSwNP).

Methods: Dispersed nasal polyp cells and uncinate tissue cells were prepared from patients with CRS with and without nasal polyps, respectively. Cells were incubated with various concentrations of alpha-toxin or staphylococcal enterotoxin B and then the levels of IL-5, IL-13, IFN-γ, IL-17A, and IL-10 in the cell supernatants were determined. The pathophysiological significance of alpha-toxin-induced cytokine production was also determined including radiological severity of rhinosinusitis, tissue and blood eosinophilia, serum total IgE level, and 1-s forced expiratory volume/forced vital capacity ratio (FEV_1/FVC).

Results: Nasal polyp cells produced substantial amounts of IL-5, IL-13, IFN-γ, IL-17A, and IL-10 in response to alpha-toxin. Cytokine production was higher in nasal polyp cells than in uncinate tissue cells. The potency of alpha-toxin in stimulating IL-5, IL-13, and IL-10 production was comparable to that of enterotoxin. Alpha-toxin-induced IFN-γ, IL-17A, and IL-10 production significantly and negatively correlated with the degree of eosinophil infiltration into nasal polyps. Conversely, alpha-toxin-induced IFN-γ and IL-10 production significantly and positively correlated with FEV_1/FVC. IL-10 production was significantly lower in asthmatic patients compared to non-asthmatics

Conclusions: *S. aureus*-derived alpha-toxin can provoke cellular responses in nasal polyps. These responses, especially failure to synthesize IL-10, may play a role in the pathophysiology of CRSwNP.

KEY WORDS

alpha-toxin, chronic rhinosinusitis, eosinophil, IL-10, nasal polyps

INTRODUCTION

Chronic rhinosinusitis with nasal polyps (CRSwNPs) is characterized by eosinophilic inflammation, and is often associated with asthma and aspirin sensitivity.[1] While the precise etiology and pathophysiology underlying this disease remains poorly understood, imbalances in local Th1, Th2, Th17, and Treg responses appear to be involved.[2,3]

Components and products derived from microbes such as viruses, fungi, and bacteria can elicit cellular responses in patients with CRSwNP.[4-7] *Staphylococcus aureus* exotoxins are among the best characterized elicitors of cellular responses and are thought to be

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heavily involved in the pathogenesis of CRSwNP through their behavior as superantigens.[4,8,9]

In addition to superantigenic exotoxins, *S. aureus* cells also contain other immunogenic components, such as the capsule, enzymes, and non-superantigenic toxins.[10,11] Since *S. aureus* per se induces an inflammatory reaction in airway cells, these components may also play a role in the pathogenesis of CRSwNP.[12]

Alpha-toxin is one of the non-superantigenic exotoxins produced by *S. aureus*.[13] Alpha-toxin is a basic protein consisting of 293 amino acids. Almost all *S. aureus* strains possess the gene that encodes alpha-toxin, *hla*. Also known as alpha-hemolysin, alpha-toxin binds to phospholipids on the surface of erythrocytes and then forms a cylindrical heptamer in the cell membrane, which induces hemolysis.[14]

Alpha-toxin can regulate immune responses in humans, including IL-1β release by monocytes, IL-8 secretion by monocytic or epithelial cell lines, LTB_4 release from neutrophils, t-bet expression and IFN-γ production by $CD4^+$ T cells, and IL-17A and IL-22 production by peripheral blood mononuclear cells.[13,15-19] The findings that *S. aureus* frequently colonizes the human nostril and that colonization by *S. aureus* is higher in patients with CRSwNP suggest that *S. aureus* alpha-toxin may play a significant role in the pathogenesis of CRSwNP.[20,21]

In the present study, we sought to determine the effect of *S. aureus* alpha-toxin on cytokine production using an ex vivo model of CRSwNP.[3] In addition, we investigated the pathophysiological significance of alpha-toxin-induced cytokine production in CRSwNP patients.

METHODS

PATIENTS

The study involved 22 Japanese CRSwNP patients (age range 26-71 years; mean age 57.8 years). The presence of CRSwNP was determined based upon diagnostic criteria reported in a European position paper on rhinosinusitis and nasal polyps.[22] All patients received a low-dose oral administration of 14-membered ring macrolides including roxithromycin and clarithromycin for at least 3 months as a basic medical therapy. Treatment failure was determined by computed tomography showing persistent abnormal shadows in sinuses. Then they received endoscopic sinus surgery. Ten patients were asthmatic, and one patient was thought to have aspirin exacerbated respiratory disease (AERD) based on a history of asthma attacks precipitated by non-steroidal anti-inflammatory drugs. For at least eight weeks prior to surgery none of the participants received systemic glucocorticoids, and for at least three weeks prior to surgery none of the participants received pharmacotherapy for rhinosinusitis, such as macrolide antibiotics or intranasal glucocorticoids. Prior to surgery, each patient's serum total IgE level (Pharmacia, Uppsala, Sweden), blood eosinophil count, and 1-s forced expiratory volume/forced vital capacity ratio (FEV_1/FVC) were determined. For ethical reason, all the asthmatic patients received inhaled corticosteroids throughout the study period including the time when the pulmonary function test was performed. A radiological assessment of the severity of rhinosinusitis in each patient was also performed using the Lund-Mackay system.[22] Sections from surgically excised nasal polyps were stained with hematoxylin/eosin solution, and the average number of eosinophils per high power field (10 × 40) was then determined.[5] Nine patients (age range 32-73 years; mean age 54.0 years) with CRS without nasal polyps (CRSsNP) were enrolled as controls.[22] Informed consent for participation in the study was obtained from each patient, and the study was approved by the Human Research Committee of the Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences.

CULTURE OF DISPERSED NASAL POLYP CELLS AND UNCINATE TISSUE CELLS

Nasal polyps and uncinate tissues were sampled from patients with CRSwNP and CRSsNP, respectively. Dispersed nasal polyp cells (DNPCs) and dispersed uncinate tissue cells (DUTCs) were prepared from nasal polyps and uncinate tissues, respectively, by enzymatic digestion, as previously described.[5] 1×10^6 DNPCs or DUTCs per mL was stimulated with 0.01, 0.1, or 1.0 ng/mL of alpha-toxin (Sigma, St. Louis, MO, USA) and incubated at 37℃ in a 5% CO_2 atmosphere. An aliquot of the culture supernatant was collected after 24 and 72 hours and stored at -80℃ for subsequent cytokine analysis. Alternatively, cells were stimulated with 1.0 ng/mL of staphylococcal enterotoxin B (SEB; Toxin Technology, Sarasota, FL, USA).

CYTOKINE MEASUREMENT

The levels of IL-5, IL-13, IFN-γ, and IL-10 were determined using Opt EIA sets (BD Biosciences, San Jose, CA, USA), according to the manufacturer's instructions. The level of IL-17A was determined using a DuoSet ELISA Development Kit (R&D Systems, Minneapolis, MN, USA). The detection limit was 4 pg/mL for IL-5, 2 pg/mL for IL-13, 4 pg/mL for IFN-γ, 8 pg/mL for IL-17A, and 4 pg/mL for IL-10.

STATISTICAL ANALYSIS

Values are given as the median. The nonparametric Mann-Whitney U test was used to compare data between groups, and Wilcoxon's signed rank test was used to analyze data within each group. Correlation analyses were performed using the Spearman rank correlation. *P*-values less than 0.05 were considered statistically significant. Statistical analyses were per-

formed with SPSS software (version 11.0, Chicago, IL, USA).

RESULTS

ALPHA-TOXIN-INDUCED CYTOKINE PRODUCTION BY DNPCs

24 h-stimulation of DNPCs with 1 ng/mL of alpha-toxin induced significant increases in the production of IL-5 (P = 0.005), IL-13 (P = 0.003), IFN-γ (P = 0.003), IL-17A (P = 0.021), and IL-10 (P < 0.001) compared to unstimulated DNPCs. Stimulation with 0.01 ng/mL of alpha-toxin did not induce production of any of the cytokines examined, while stimulation with 0.1 ng/mL did induce significant production of IL-10 (P = 0.003). In addition, 72 h-stimulation of DNPCs with 0.01 ng/mL alpha-toxin resulted in a significant production of all the cytokines tested, and these productions were substantially increased in a dose-dependent manner (Fig. 1; P < 0.001).

COMPARISON OF ALPHA-TOXIN-INDUCED CYTOKINE PRODUCTION BY NASAL POLYP CELLS AND UNCINATE TISSUE CELLS

Stimulation of 1 × 10^6/mL of uncinate tissue cells derived from CRSsNP patients with 1 ng/mL of alpha-toxin for 72 h induced a modest but significant increase in production of IL-5 (P = 0.043), IL-13 (P = 0.006), IFN-γ (P = 0.012), IL-17A (P = 0.012), and IL-10 (P = 0.018). However, as shown in Figure 2, stimulating the same number of nasal polyp cells derived from CRSwNP patients in the same manner induced production of significantly higher levels of these cytokines (IL-5: P = 0.001, IL-13: P = 0.017, IFN-γ: P = 0.006, IL-17A: P = 0.004, IL-10: P = 0.001).

COMPARISON OF CYTOKINE PRODUCTION FOLLOWING STIMULATION WITH ALPHA-TOXIN OR SEB

Next, we compared the potency of alpha-toxin and SEB, with respect to inducing cytokine production by DNPCs. Stimulation with alpha-toxin induced production of a similar amount of IL-5 (P = 0.230), IL-13 (P = 0.516), and IL-10 (P = 0.263) as did stimulation with SEB (Fig. 3A, B, E). In contrast, production of IFN-γ (P = 0.002) and IL-17A (P < 0.001) was significantly lower in DNPCs stimulated with alpha-toxin than in DNPCs stimulated with SEB (Fig. 3C, D).

PATHOPHYSIOLOGICAL SIGNIFICANCE OF ALPHA-TOXIN-INDUCED CYTOKINE PRODUCTION IN CRSwNP

As shown in Table 1, the production of IL-5 (ρ = 0.146, P = 0.503) and IL-13 (ρ = 0.108, P = 0.621) following a 72-h stimulation with 1 ng/mL of alpha-toxin did not correlate with eosinophil infiltration into nasal polyps. However, there was a significant negative correlation between local eosinophilia and production of IFN-γ (ρ = -0.432, P = 0.048), IL-17A (ρ = -0.566, P = 0.009), and IL-10 (ρ = -0.567, P = 0.009) (Fig. 4). Alpha-toxin-induced cytokine production did not correlate with other pathophysiological parameters, including radiological severity of rhinosinusitis, blood eosinophil count, or serum total IgE or FEV_1/FVC, except for positive correlations between IFN-γ production and FEV_1/FVC (ρ = 0.456, P = 0.037) and between IL-10 production and FEV_1/FVC (ρ = 0.538, P = 0.014). Production of IL-10 was significantly lower in asthmatic patients compared to non-asthmatics (P = 0.035); however, the presence of asthma did not affect the production of other cytokines (Fig. 5).

DISCUSSION

In the present study, we characterized the alpha-toxin-induced cytokine productions from patients with CRSwNP and CRSsNP. Our results suggest that antigens other than SEB, namely alpha-toxin in this study, are also likely targets for stimulatory molecules. Both the innate and the adaptive immune systems clearly respond to bacterial structural components as well as their secreted exotoxins.[4] In this case, alpha-toxin can stimulate a robust synthesis of cytokines known to be associated with eosinophilic airway inflammation.

In response to alpha-toxin stimulation, DNPCs produce substantial amounts of IL-5, IL-13, IFN-γ, IL-17A, and IL-10, cytokines known to regulate the pathogenesis of CRSwNP.[2,3] In nasal polyps, a majority of the IL-5-producing cells is T cells, and mast cells and eosinophils can express IL-5.[23] Mononuclear cells and eosinophils in nasal polyps express IL-13.[24,25] We have previously reported that macrophages, $CD4^+$ T cells and eosinophils express IL-17A in nasal polyps.[3] T cells are the principal source of allergen-induced IL-10 production in nasal polyps.[26] Thus, alpha-toxin may stimulate these inflammatory cells directly and/or indirectly to produce cytokines. Additional experiments, e.g. whether adherent cell or non-adherent cell produce cytokines in response to alpha-toxin, should be performed in order to identify which cells are the main sources for cytokine production.

High concentrations of alpha-toxin induce cell death due to the formation of hexameric transmembrane pores.[13] For example, about two-thirds of Jurkat T cells treated with 100 ng/mL of alpha-toxin will undergo subsequent apoptosis.[27] In contrast, exposure to lower concentrations of alpha-toxin can induce pro-inflammatory effects in a variety of cells without causing cell lysis, presumably via alteration of the cellular ion balance following pore formation, particularly through calcium influx.[16] For example, exposure of human $CD4^+$ T cells to alpha-toxin at concentrations less than 100 ng/mL does not lead to cell death.[16] Alpha-toxin concentrations less than 1,000 ng/mL were found to be sublytic to human macrophages.[19] In the present study, we exposed DNPCs and DUTCs to alpha-toxin at concentrations of 0.01,

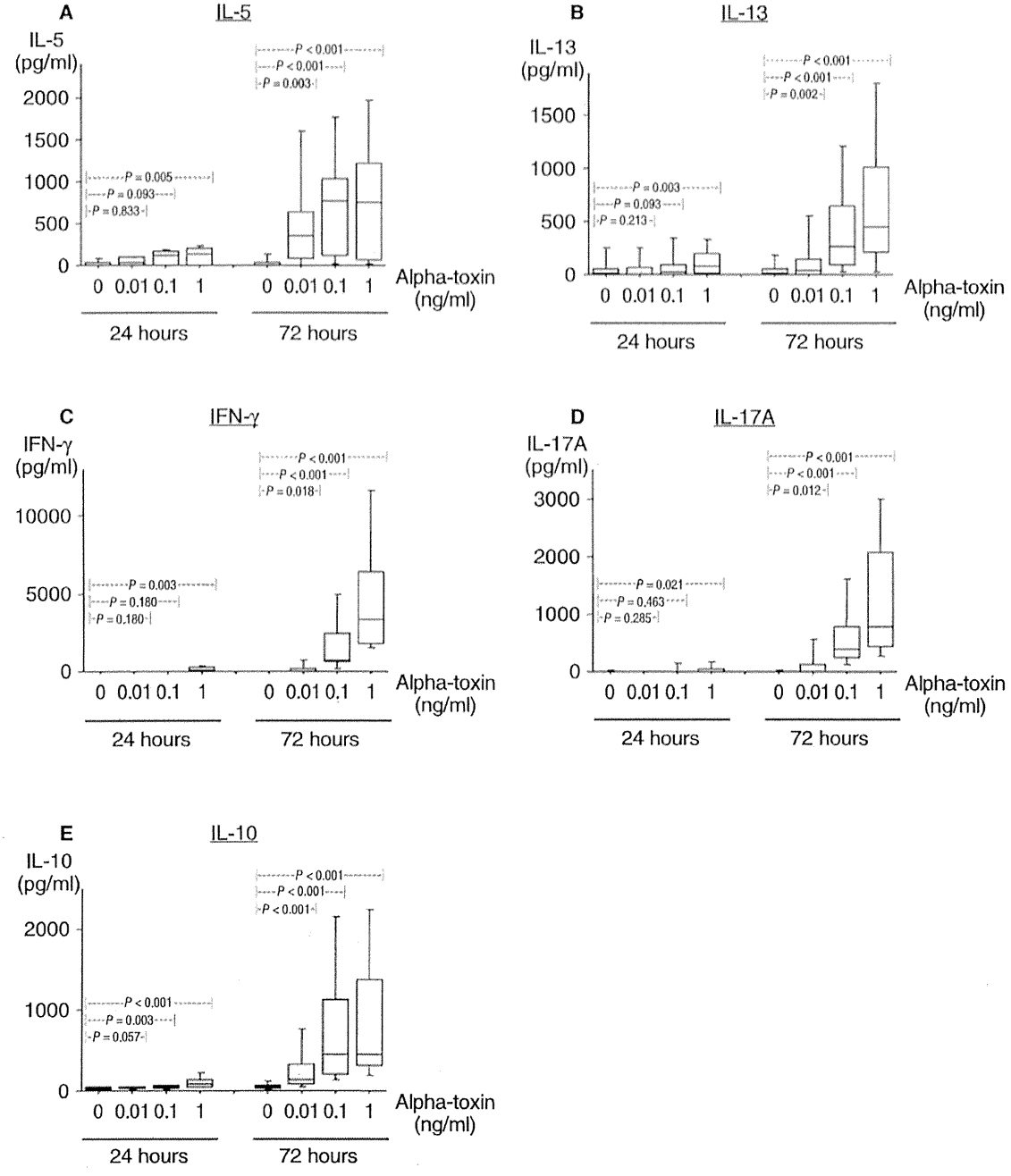

Fig. 1 Effect of alpha-toxin of *S. aureus* on IL-5 (**A**), IL-13 (**B**), IFN-γ (**C**), IL-17A (**D**) and IL-10 (**E**) production by DNPCs (n = 22). DNPCs were stimulated with 0.01, 0.1, or 1.0 ng/mL of alpha-toxin and incubated for either 24 or 72 hours. The rectangle includes the range from the 25th to the 75th percentiles, the horizontal line indicates the median, and the vertical line indicates the range from the 10th to the 90th percentiles. P values were determined by Wilcoxon's signed-ranks test.

0.1, and 1.0 ng/mL, concentrations which were lower than those used in previous studies.[15-19] Our preliminary study revealed that 1 ng/mL of alpha-toxin does not induce a substantial number of cells to undergo cell death, as determined by trypan blue dye exclusion tests (data not shown). These results suggest that in CRSwNP alpha-toxin induces active production of cytokines rather than their release due to cell lysis.

It seems to be difficult to determine the actual concentration of alpha-toxin in the nose because viable *S. aureus* may consistently produce the toxin. In addition, kits to determine the concentration is not commercially available at present. Since hemolysis of erythrocytes is not generally detected in nasal polyps, we think that the concentration of alpha-toxin in the

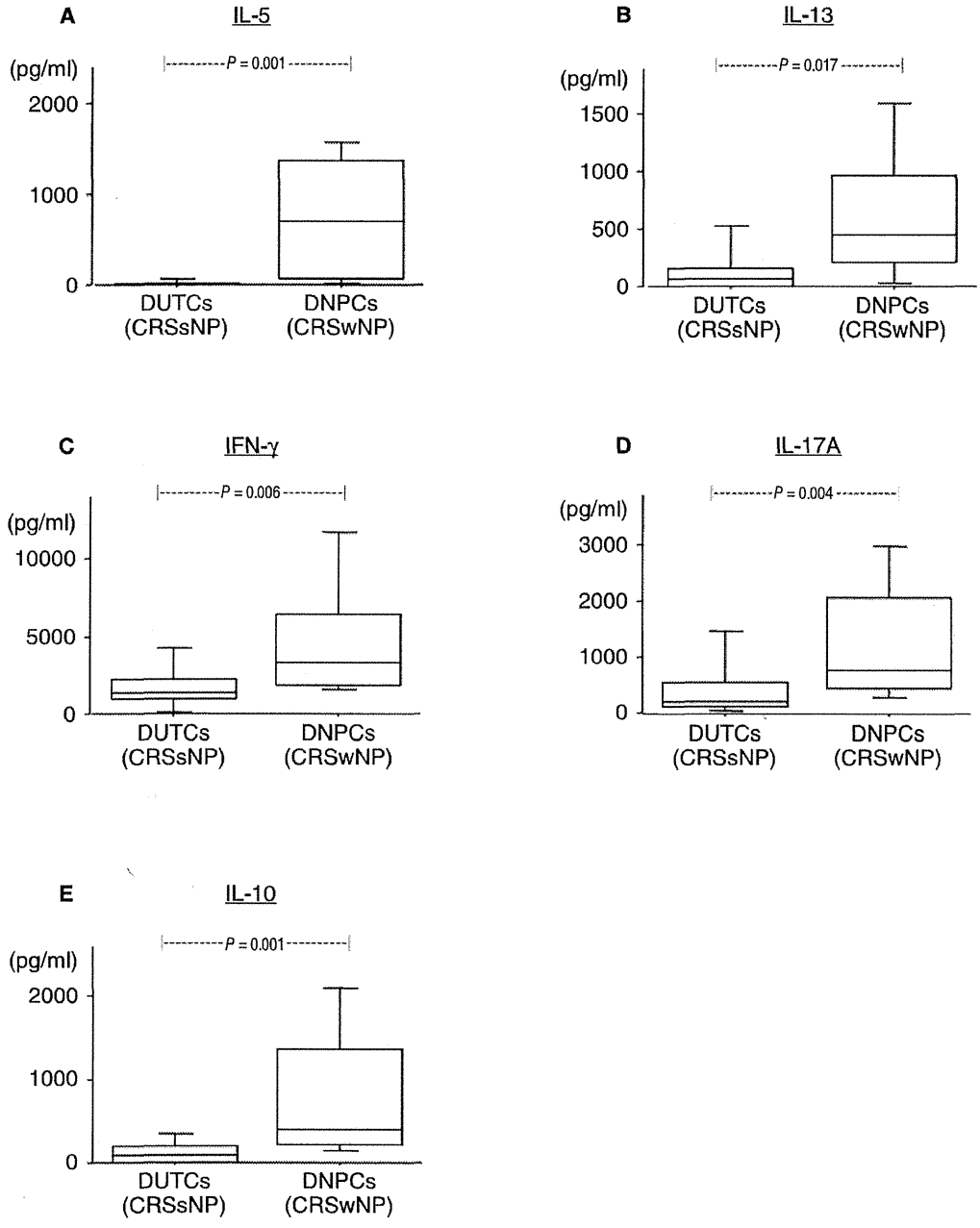

Fig. 2 Comparison of alpha-toxin-induced IL-5 (**A**), IL-13 (**B**), IFN-γ (**C**), IL-17A (**D**) and IL-10 (**E**) production between DNPCs from patients with CRSwNP (n = 22) and DUTCs from patients with CRSsNP (n = 9). The rectangle includes the range from the 25th to the 75th percentiles, the horizontal line indicates the median, and the vertical line indicates the range from the 10th to the 90th percentiles. P values were determined by Mann-Whitney's U test.

mucus adjacent to the polyps is below 200 nM.[14] 1 ng/ml (approximately 30 pM) of alpha-toxin could induce cytokine production including IL-5, IL-13, IFN-γ and IL-17A by nasal polyp cells, suggesting that alpha-toxin is being produced in sufficient concentrations that would produce a pro-inflammatory effect in the polyp tissue.

In response to alpha-toxin stimulation, nasal polyps cells from CRSwNP produced significantly higher amounts of IL-5, IL-13, IFN-γ, IL-17A, and IL-10 than did uncinate tissue cells from CRSsNP. This result was similar to the previous report by Patou *et al.*, who demonstrated that SEB stimulated significantly higher production of IL-2, IL-4, IL-5, IL-10, IL-13, and IFN-γ in nasal polyps than in inferior turbinates.[4] Our results suggest that, as is the case with SEB, alpha-toxin induces enhanced immune responses that are associated with nasal polyp formation in CRSwNP.

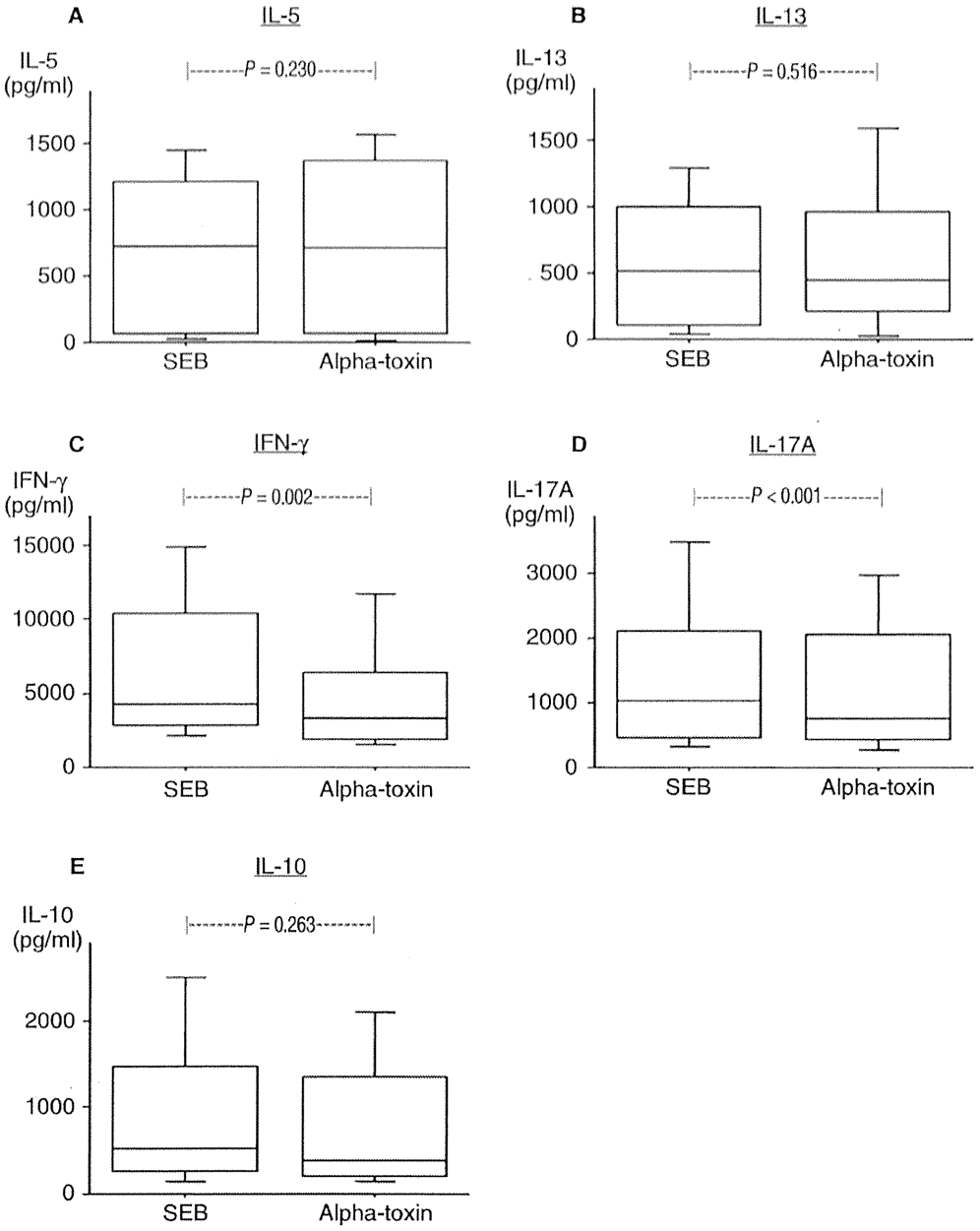

Fig. 3 Comparison of IL-5 (**A**), IL-13 (**B**), IFN-γ (**C**), IL-17A (**D**) and IL-10 (**E**) production by DNPCs between alpha-toxin and SEB stimulation. DNPCs (n = 22) were stimulated with 1.0 ng/mL of either alpha-toxin or SEB, and incubated for 72 hours. Bar indicates the median. The rectangle 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 the 90th percentiles. P values were determined by Wilcoxon's signed-ranks test.

The introduction of a healthy control cohort would bring more clarity into the project, and our preliminary results showed that the dispersed cells from non-inflamed concha bullosa (n = 3) produced less amounts of IL-5 (73.3 pg/ml), IL-13 (160.3 pg/ml), IFN-γ (766.7 pg/ml), IL-17A (84.3 pg/ml), and IL-10 (147.7 pg/ml) as compared with DNPCs. Although substantial cases of nasal polyps are grown from untinate tissue, future examination about the comparison of alpha-toxin-induced cytokine production between uncinate tissue cells and nasal polyp cells in identical patients with CRSwNP or AERD will provide valuable information for understanding the pathogenesis of this disease such as the mechanisms of nasal polyp formation.

We previously reported that exposure to SEB at a concentration of 1 ng/mL induces IL-5, IL-13, IFN-γ, and IL-17A production in DNPCs.[3,28] In the present

Table 1 Correlation between pathophysiological characterizations and alpha-toxin-induced cytokine production by DNPCs

		Nasal polyp eosinophilia (cells/field)	Radiological severity (score)	Blood eosinophilia (cells/μl)	Serum total IgE (IU/ml)	FEV_1/FVC
IL-5	ρ	0.146	0.103	0.411	-0.089	0.041
	P	0.503	0.646	0.060	0.685	0.850
IL-13	ρ	0.108	0.111	0.278	-0.307	0.074
	P	0.621	0.620	0.202	0.160	0.735
IFN-γ	ρ	-0.432	-0.097	-0.263	-0.408	0.456
	P	0.048	0.644	0.229	0.061	0.037
IL-17A	ρ	-0.566	-0.014	-0.372	-0.192	0.298
	P	0.009	0.937	0.088	0.377	0.172
IL-10	ρ	-0.567	-0.334	-0.295	-0.250	0.538
	P	0.009	0.121	0.176	0.252	0.014

ρ and *P* values were determined by Spearman's rank correlation coefficient.

study, we also demonstrated that exposing DNPCs to SEB induces IL-10 production. This result was in agreement with the previous report by Patou *et al.*, which showed that 500 ng/mL of SEB induces IL-10 production in nasal polyp explants.[3] We found that at 1 ng/mL, both alpha-toxin and SEB induce production of similar amounts of IL-5, IL-13, and IL-10 in DNPCs. Our preliminary experiment showed that simultaneous exposure to alpha-toxin and SEB did not display a synergistic effect on cytokine production by DNPCs as compared with the single exposure. Since the molecular weight of SEB (approximately 27 kDa) is similar to that of alpha-toxin (28-30 kDa), these results suggest that both the superantigenic and non-superantigenic exotoxins of *S. aureus* equally induce Th2 and regulatory immune responses in CRSwNP.[14,29] However, we found that production of IFN-γ and IL-17A is significantly lower in DNPCs exposed to alpha-toxin than in cells exposed to SEB. Other studies have shown that IFN-γ and IL-17A play roles in the pathogenesis of CRS.[3,30] For example, IFN-γ is abundantly expressed in CRSsNP as compared with CRSwNP.[30] These results suggest that SEB affects cytokine production more broadly than does alpha-toxin, probable due to its superantigenic profile.

There was a significant negative correlation between the degree of eosinophil infiltration into nasal polyps and alpha-toxin-induced production of IFN-γ, IL-17A, and IL-10 by DNPCs. Studies involving mice have shown that IFN-γ inhibits airway eosinophilia by blocking Th2 cell cytokine production, $CD4^+$ T cell recruitment into airways, and/or by suppressing the function of antigen-presenting cells.[31,32] In humans, 80% of mild asthmatics that received IFN-γ via inhalation exhibited a reduction in the number of airway eosinophils.[33] IFN-γ causes an opening of tight junctions in CRS which may lead to shedding and drainage of inflammation.[34] The present results consist with the reports, and suggest that IFN-γ has a regulatory effect on eosinophilic inflammation in CRSwNP.[34] In contrast, alpha-toxin-induced production of IL-5 and IL-13 did not correlate with local eosinophilia. This result was similar to those of our previous report, which found no correlation between SEB-induced production of IL-5 and IL-13 and local eosinophilia.[5] However, as shown in Table 1, the levels of IL-5 production showed a tendency to positively correlate with blood eosinophilia ($\rho = 0.411$, $P = 0.060$). IL-5 is involved in the differentiation and maturation of eosinophils in the bone marrow, migration to tissue sites, and prevention of eosinophil apoptosis.[35] Our results suggest that alpha-toxin-induced IL-5 production is involved in the pathophysiology of CRSwNP by increasing blood eosinophils. In fact, a recent study showed that IFN-γ suppresses airway eosinophilia independent of Th2 cell activation.[32]

It is known that IL-10 plays a critical role in controlling eosinophilic airway inflammation.[36] In CRS patients, IL-10 suppresses IL-6 and TNF-α production by upper airway dendritic cells.[37] Additionally, IL-10 suppresses allergen-induced IL-5 and IFN-γ production by DNPCs.[32] In the present study, the negative correlation between the degree of eosinophil infiltration into nasal polyps and alpha-toxin-induced cytokine production was strongest for IL-10. These results suggest that alpha-toxin-induced IL-10 production plays an important role in protecting eosinophilic inflammation in CRSwNP.

It remains unclear why IL-10 production is suppressed in DNPCs derived from asthmatic patients. However, a recent meta-analysis demonstrated the association between IL-10 promoter gene polymorphism and susceptibility to asthma.[38] Another study showed a lower serum concentration of IL-10 in patients with early-onset current asthma.[39] Thus asthmatic patients may genetically show the impairment in IL-10 production even in the upper airway.

Alpha-toxin-induced IL-10 production was positively correlated with FEV_1/FVC. This may be due to the inhibitory effect of IL-10 on eosinophilic inflammation by suppression of IL-5 and GM-SCF through

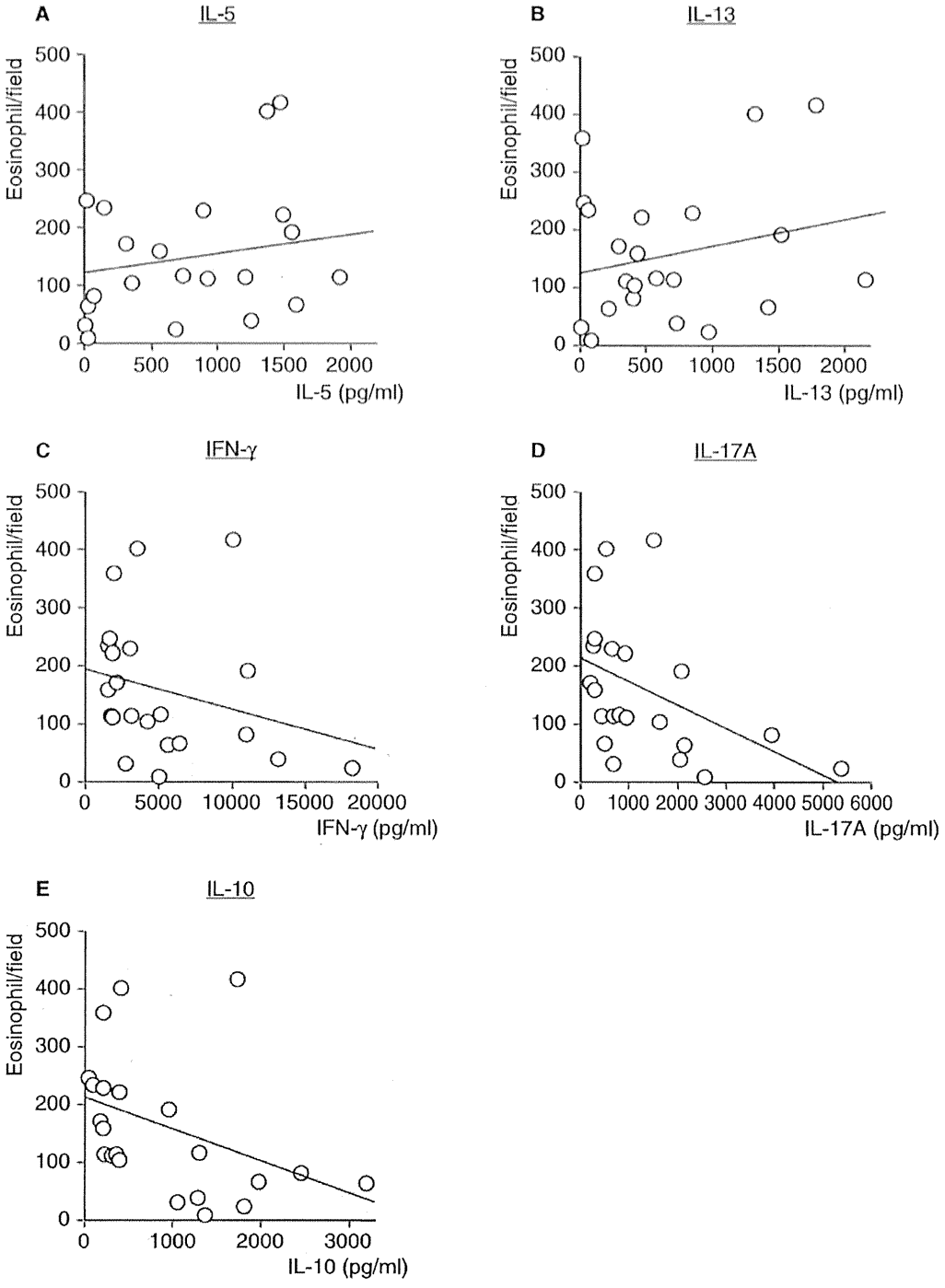

Fig. 4 Relationship between the number of eosinophils in nasal polyps and alpha-toxin-induced IL-5 (**A**), IL-13 (**B**), IFN-γ (**C**), IL-17A (**D**) and IL-10 (**E**) production by DNPCs ($n = 22$).

upregulation of suppressor of cytokine signaling (SOCS)-3.[40] Alternatively, suppression of airway nitric oxide production by IL-10 may be involved the function.[40]

AERD is one of endotypes in CRSwNP.[41] Amount of alpha-toxin (1 ng/ml)-induced IL-5 (883 pg/ml), IL-13 (849 pg/ml), IFN-γ (2,951 pg/ml), IL-17A (627 pg/ml) and IL-10 (200 pg/ml) by DNPC from a patient with AERD was not an outlier of the average +/- standard deviation of IL-5 (752 +/- 640 pg/ml), IL-13 (670 +/- 615 pg/ml), IFN-γ (5,249 +/- 4,583 pg/ml), IL-17A (1,305 +/- 1,312 pg/ml) and IL-10 (905 +/- 871) of 22 DNPCs, respectively, as determined by Grubbs test ($P > 0.05$). This suggests that the data from a patient with AERD had little effect on skewing the results.

In conclusion, not only superantigenic but also non-superantigenic exotoxins derived from *S. aureus* modulate local immune reactions and affect the

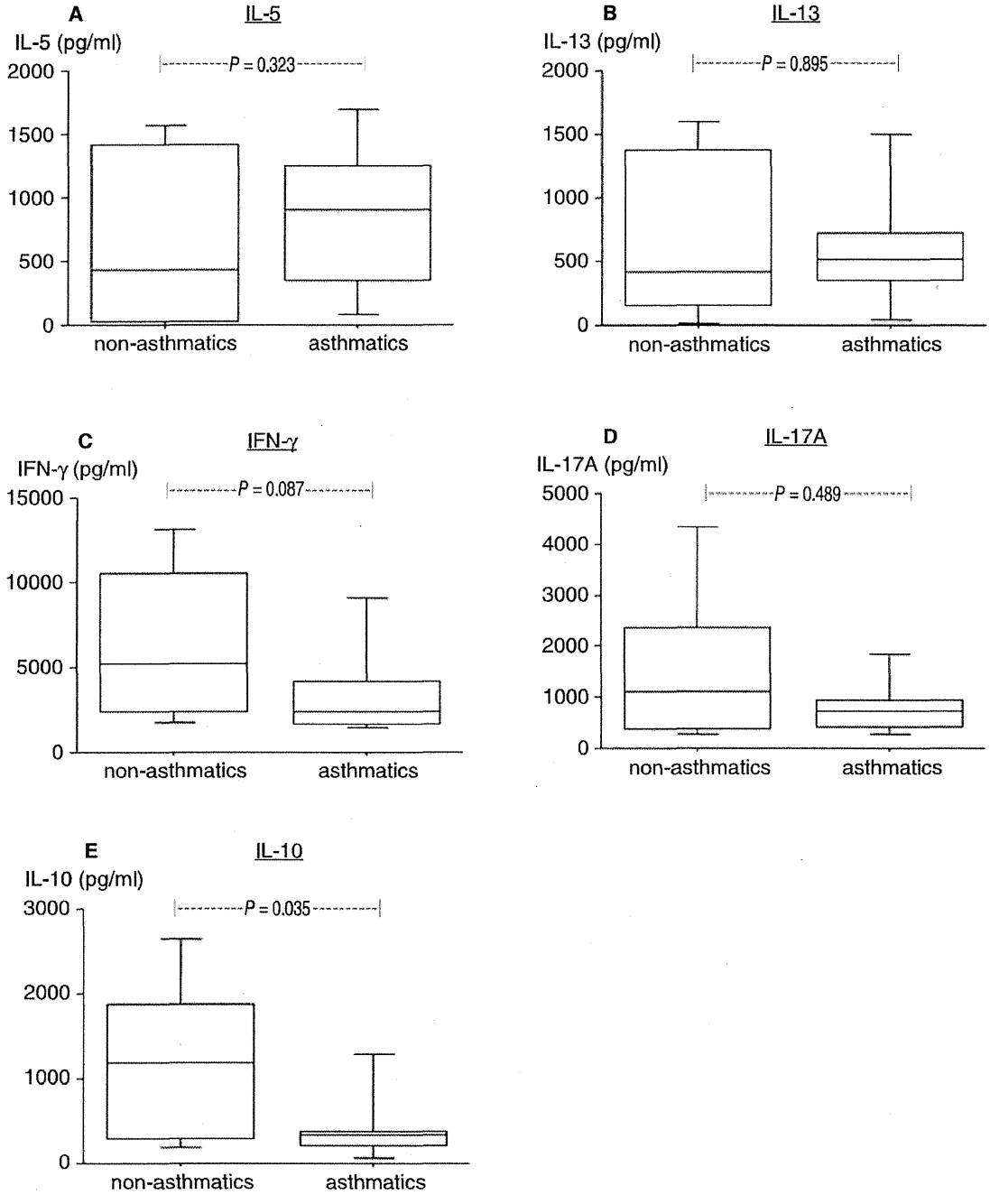

Fig. 5 Comparison of alpha-toxin-induced IL-5 (**A**), IL-13 (**B**), IFN-γ (**C**), IL-17A (**D**) and IL-10 (**E**) production by DNPCs between non-asthmatic (n = 11) and asthmatic (n = 10) patients. The rectangle includes the range from the 25th to the 75th percentiles, the horizontal line indicates the median, and the vertical line indicates the range from the 10th to the 90th percentiles. P values were determined by Mann-Whitney's U test.

pathophysiology of CRSwNP. It is known that the endoscopic sinus surgery can improve pulmonary function in asthmatic patients.[42,43] In addition, Proimos *et al.* showed a clear improvement in the use of bronchodilators, oral steroids, and need for hospitalization for asthma after the surgery in asthmatic CRS patients.[44] The present findings may provide new insight into the role of alpha-toxin in the pathogenesis of eosinophilic airway diseases, including allergic rhinitis and bronchial asthma, and provide a basis for the development of novel therapeutic approaches that target *S. aureus* in order to limit eosinophilic airway inflammation.