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Chronic rhinosinusitis patients have decreased lung function

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Background: The relationship between upper and lower airway diseases has been reported. However, the pulmonary function of patients with chronic rhinosinusitis (CRS) has not been fully examined.

Methods: Pulmonary function was measured in 273 patients with CRS and 100 age-matched normal control subjects. No patients with chronic obstructive pulmonary disease (COPD) were included in this study. The patients with CRS were divided into 8 subgroups based on the presence of asthma, sensitization to common inhaled antigens, and nasal polyposis. The relationships between pulmonary function and clinical parameters, including radiographic severity of CRS according to the Lund-Mackay computed tomography (CT) staging system, eosinophil count in the peripheral blood, and serum total immunoglobulin E (IgE) levels, were assessed.

Results: In pulmonary function testing, the CRS patients had affected pulmonary function. The CRS patients without asthma showed latent obstructive pulmonary function changes when compared to normal controls. No significant

correlations were observed between pulmonary function and any clinical parameters (Lund-Mackay CT staging score, eosinophil count in the peripheral blood, and serum total IgE levels).

Conclusion: CRS patients had significant obstructive lung function changes regardless of the presence of asthma. The patients with CRS who had not been clinically diagnosed as having lower respiratory tract diseases might have had subclinical lower airway diseases. Therefore, clinicians should be aware of pulmonary function and lower lung diseases in patients with CRS. © 2014 ARS-AAOA, LLC.

Key Words:

sinusitis; rhinitis; asthma; chronic obstructive pulmonary disease; pulmonary function

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A close link between upper and lower respiratory disease has been reported.¹ Chronic rhinosinusitis (CRS) and rhinitis are both common upper respiratory tract conditions among patients with asthma and chronic obstructive pulmonary disease (COPD).^{2,3} The impact of allergic rhinitis on asthma is well known; however, the effects of the affected paranasal sinus in lower airway disease manifestations remains largely unclear.¹ Although numerous studies have reported a pathophysiologic link between allergic upper and lower airway disease, the relationship between nonallergic sinus disease and asthma or COPD has not yet been fully understood.⁴

Patients with asthma or COPD are known to have decreased pulmonary function; however, the pulmonary function of patients with upper respiratory tract disease has not been elucidated. Therefore, the present study aimed to characterize the pulmonary function of CRS patients, especially those without asthma, in comparison to healthy subjects.

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Patients and methods

Subjects

A total of 273 patients with CRS who received endoscopic sinus surgery at Okayama University were recruited. The diagnosis of CRS was based on the definition in European Position Paper on Rhinosinusitis and Nasal Polyps 2012.⁵ Among these 273 patients with CRS, 70 patients had

asthma (57 patients with nasal polyposis; 13 patients without nasal polyposis) and 203 patients were subjects with no previous diagnosis of asthma (135 patients with nasal polyposis; 68 patients without nasal polyposis).

All CRS patients were resistant to medical treatment, including macrolide therapy.⁶ CRS patients with COPD were excluded from this study.

Age-matched normal control subjects ($n = 100$) without any chronic respiratory diseases were also recruited. Because cigarette smoking affects pulmonary function, smoking status and the Brinkman index (number of cigarettes per day \times number of years of smoking) were examined. Informed consent was obtained from enrolled subjects. The protocol of this study was approved by the Institutional Review Board of Okayama University and all procedures were carried out in compliance with the Declaration of Helsinki of 1995 (as revised in 2008).

Pulmonary function testing

Before endoscopic sinus surgery, pulmonary function testing was performed with the CHESTAC-9800 (CHEST M.I., Inc., Tokyo, Japan) according to the standardization of pulmonary function tests of the American Thoracic Society and European Respiratory Society.⁷ The following parameters were measured or calculated: percent predicted vital capacity (%VC), forced expiratory volume in 1 second ($FEV_{1.0}$), percent predicted $FEV_{1.0}$ (% $FEV_{1.0}$), $FEV_{1.0}$ /forced vital capacity ratio ($FEV_{1.0}/FVC$ ratio), mean forced expiratory flow between 25% and 75% of the forced vital capacity ($FEF_{25\%-75\%}$), peak expiratory flow (PEF), maximal expiratory flow rate at 50% of vital capacity (V_{50}), maximal expiratory flow rate at 25% of vital capacity (V_{25}), and the V_{50}/V_{25} ratio.

CRS assessment by computed tomography staging

The radiographic severity of CRS was assessed according to the Lund-Mackay computed tomography (CT) staging system before endoscopic sinus surgery.⁸

Peripheral blood eosinophil count, total immunoglobulin E (IgE) level, and specific IgE level in serum samples

Blood samples were collected before endoscopic sinus surgery. The eosinophil count in the peripheral blood was determined. In serum samples, the total IgE and specific IgE to 13 allergens (*Dermatophagoides pteronyssinus*, *Felis domesticus*, *Canis familiaris*, *Dactylis glomerata*, *Aspergillus fumigatus*, *Candida albicans*, *Alternaria alternata*, *Trichophyton rubrum*, *Alnus incana*, *Cupressus japonica*, *Chamaecyparis obtusa*, *Ambrosia elatior*, and *Artemisia vulgaris*) were measured using the ImmunoCap 250 system (Phadia AB, Uppsala, Sweden) according to the manufacturer's protocols.

TABLE 1: Clinical parameters of pulmonary function in patients with chronic rhinosinusitis and normal control subjects

| | Chronic rhinosinusitis with asthma | Chronic rhinosinusitis without asthma | Control |
|-----------------------|------------------------------------|---------------------------------------|------------------|
| Subjects (n) | 70 | 203 | 100 |
| %VC | 110.1 \pm 16.5 | 112.6 \pm 15.8 | 113.5 \pm 11.7 |
| $FEV_{1.0}$ | 2.4 \pm 0.9* | 3.3 \pm 0.8 | 3.3 \pm 0.7 |
| % $FEV_{1.0}$ | 89.0 \pm 19.2* | 100.3 \pm 15.5* | 105.9 \pm 10.5 |
| $FEV_{1.0}/FVC$ ratio | 68.9 \pm 10.9* | 81.6 \pm 8.3* | 85.2 \pm 6.3 |
| $FEF_{25\%-75\%}$ | 1.57 \pm 1.04* | 3.06 \pm 1.26* | 3.70 \pm 1.11 |
| PEF | 6.30 \pm 2.08* | 7.87 \pm 1.88* | 8.45 \pm 2.27 |
| V_{50} | 1.90 \pm 1.18* | 3.63 \pm 1.32* | 4.19 \pm 1.14 |
| V_{25} | 0.61 \pm 0.47* | 1.42 \pm 0.80* | 1.71 \pm 0.84 |
| V_{50}/V_{25} ratio | 3.68 \pm 1.79* | 3.06 \pm 1.64* | 2.75 \pm 0.86 |

Data represent means \pm standard deviation.

* $p < 0.05$ as compared with normal control.

% $FEV_{1.0}$ = percent predicted $FEV_{1.0}$; %VC = percent predicted vital capacity; $FEF_{25\%-75\%}$ = mean forced expiratory flow between 25% and 75% of the forced vital capacity; $FEV_{1.0}$ = forced expiratory volume in one second; FVC = forced vital capacity; PEF = peak expiratory flow; V_{25} = maximal expiratory flow rate at 25% of vital capacity; V_{50} = maximal expiratory flow rate at 50% of vital capacity.

Statistical analysis

All values are presented as means \pm standard deviation. Differences in proportions were tested by the chi-square test. For comparisons between 3 or more groups, a 1-way analysis of variance (ANOVA) was performed to establish the significance of intergroup variability, and 2-tailed unpaired t tests were then used for between-group comparisons. Correlations were analyzed using Pearson's correlation coefficient. Values of $p < 0.05$ were considered statistically significant. All statistical analyses were performed using IBM SPSS statistical software (IBM, Armonk, NY).

Results

Pulmonary function in CRS patients with and without asthma

The pulmonary function of CRS patients with and without asthma and normal control subjects is shown in Table 1. No significant difference in %VC was observed between CRS patients and normal controls. In CRS patients with asthma, $FEV_{1.0}$ was significantly smaller than in CRS patients without asthma and normal control subjects ($p < 0.05$). There were statistically significant differences in % $FEV_{1.0}$, $FEV_{1.0}/FVC$ ratio, $FEF_{25\%-75\%}$, PEF, V_{50} , V_{25} , and V_{50}/V_{25} ratio among all 3 groups ($p < 0.05$). Impaired pulmonary function was observed even in CRS patients without asthma or COPD.

TABLE 2. The characteristics of patients and normal controls

| | Chronic rhinosinusitis with asthma | | | | Chronic rhinosinusitis without asthma | | | | | |
|------------------------------------|------------------------------------|---------------|---------------|--------------|---------------------------------------|---------------|---------------|---------------|---------------|---------|
| Sensitization to inhaled allergens | Positive | | Negative | | Positive | | Negative | | | |
| Nasal polyposis | Present | Absent | Present | Absent | Present | Absent | Present | Absent | Control | p |
| Subjects (n) | 48 | 8 | 9 | 5 | 98 | 55 | 37 | 13 | 100 | |
| Gender (male/female) (n) | 29/19 | 4/4 | 3/6 | 2/3 | 66/32 | 36/19 | 25/12 | 5/8 | 51/49 | 0.101* |
| Age (years) | 44.0 ± 10.8 | 38.7 ± 10.2 | 47.4 ± 11.4 | 46.7 ± 6.4 | 36.9 ± 11.8 | 39.4 ± 11.8 | 38.8 ± 12.4 | 40.1 ± 10.0 | 38.7 ± 10.8 | 0.053** |
| Smoking status (n) | | | | | | | | | | 0.103* |
| Past smoker | 17 | 2 | 2 | 2 | 19 | 9 | 7 | 3 | 25 | |
| Current smoker | 3 | 1 | 0 | 0 | 27 | 15 | 13 | 5 | 20 | |
| Nonsmoker | 28 | 5 | 7 | 3 | 52 | 31 | 17 | 5 | 55 | |
| Brinkman index | | | | | | | | | | |
| All smoker | 540.5 ± 477.4 | 256.7 ± 127.4 | 105.0 ± 134.4 | 280.0 ± 56.6 | 349.2 ± 273.1 | 462.0 ± 317.0 | 462.7 ± 364.5 | 366.9 ± 292.8 | 313.2 ± 289.3 | 0.156** |
| Past smoker | 568.8 ± 487.0 | 330.0 ± 14.1 | 105.0 ± 134.4 | 280.0 ± 56.6 | 333.2 ± 282.7 | 433.6 ± 352.7 | 432.9 ± 323.9 | 220.0 ± 163.7 | 307.0 ± 301.5 | 0.347** |
| Current smoker | 379.7 ± 471.8 | 100 | N/A | N/A | 360.5 ± 271.0 | 479.0 ± 305.2 | 478.8 ± 396.4 | 455.0 ± 332.7 | 321.0 ± 280.9 | 0.600** |

Data represent means ± standard deviation.
*Chi-square test.
**One-way analysis of variance
Brinkman index = number of cigarettes per day × number of years of smoking; N/A = not applicable.

Effect of sensitization to inhaled allergens and nasal polyposis on pulmonary function

As allergic status and nasal polyposis might have a critical effect on pulmonary function, we grouped the patients with CRS based on sensitization to common inhaled antigens and nasal polyposis. The patients were defined as sensitized if specific IgE levels were equal to or greater than 0.35 U_A/mL for at least 1 of the 13 inhaled antigens.

The clinical characteristics of the subjects are summarized in Table 2. There were no significant age differences among the groups. The current smoking rate in asthmatic CRS patients (sensitization positive, 7.1%, 4/56; sensitization negative, 0%, 0/14) was smaller than in nonasthmatic CRS patients and in normal control subjects. No statistically significant difference in smoking habits and the Brinkman index was observed between the groups.

The pulmonary function of each of the 9 groups is shown in Figure 1. No significant difference in %VC was observed between CRS patients and normal controls (Fig. 1A). When compared with normal controls, the asthmatic patients showed affected pulmonary function in FEV_{1.0}, %FEV_{1.0}, FEV_{1.0}/FVC ratio, FEF_{25%-75%}, PEF, V₅₀, V₂₅, and V₅₀/V₂₅ ratio, regardless of sensitization status (Fig. 1B-I). Significant differences in %FEV_{1.0}, FEV_{1.0}/FVC ratio, FEF_{25%-75%},

V₅₀, and V₂₅ were observed between the nonasthmatic CRS patients and normal controls (Fig. 1C-E, G, H).

Serum total IgE levels

Serum total IgE levels of the subjects are summarized in Table 3. The CRS patients with sensitization had a significantly higher IgE level compared with the CRS patients without sensitization (*p* < 0.05). No statistically significant correlation was observed between serum total IgE levels and lung function.

Peripheral blood eosinophil count

Peripheral blood eosinophil counts of the subjects are summarized in Table 3. Nonasthmatic CRS patients with sensitization and nasal polyps and asthmatic patients had a significantly higher eosinophil count when compared with normal control subjects (*p* < 0.05). No statistically significant correlation was observed between peripheral blood eosinophil count and lung function.

The Lund-Mackay CT score

The Lund-Mackay CT scores of the subjects are summarized in Table 3. The CRS patients with nasal polyposis had significantly higher Lund-Mackay CT scores compared to

the CRS patients without nasal polyposis ($p < 0.05$). No statistically significant correlation was observed between Lund-Mackay CT score and lung function.

Discussion

CRS is characterized by inflammation of the nose and paranasal sinuses of unknown cause defined on the

basis of characteristic symptoms (2 or more of the following: nasal congestion, facial pain/pressure, anterior or posterior nasal drainage, and reduced or absent sense of smell), duration (more than 12 weeks), and objective evidence of sinus disease by means of direct visualization or imaging studies. CRS is a common disease, and affects 12.5% of the U.S. population or approximately 31 million patients each year.^{9,10}

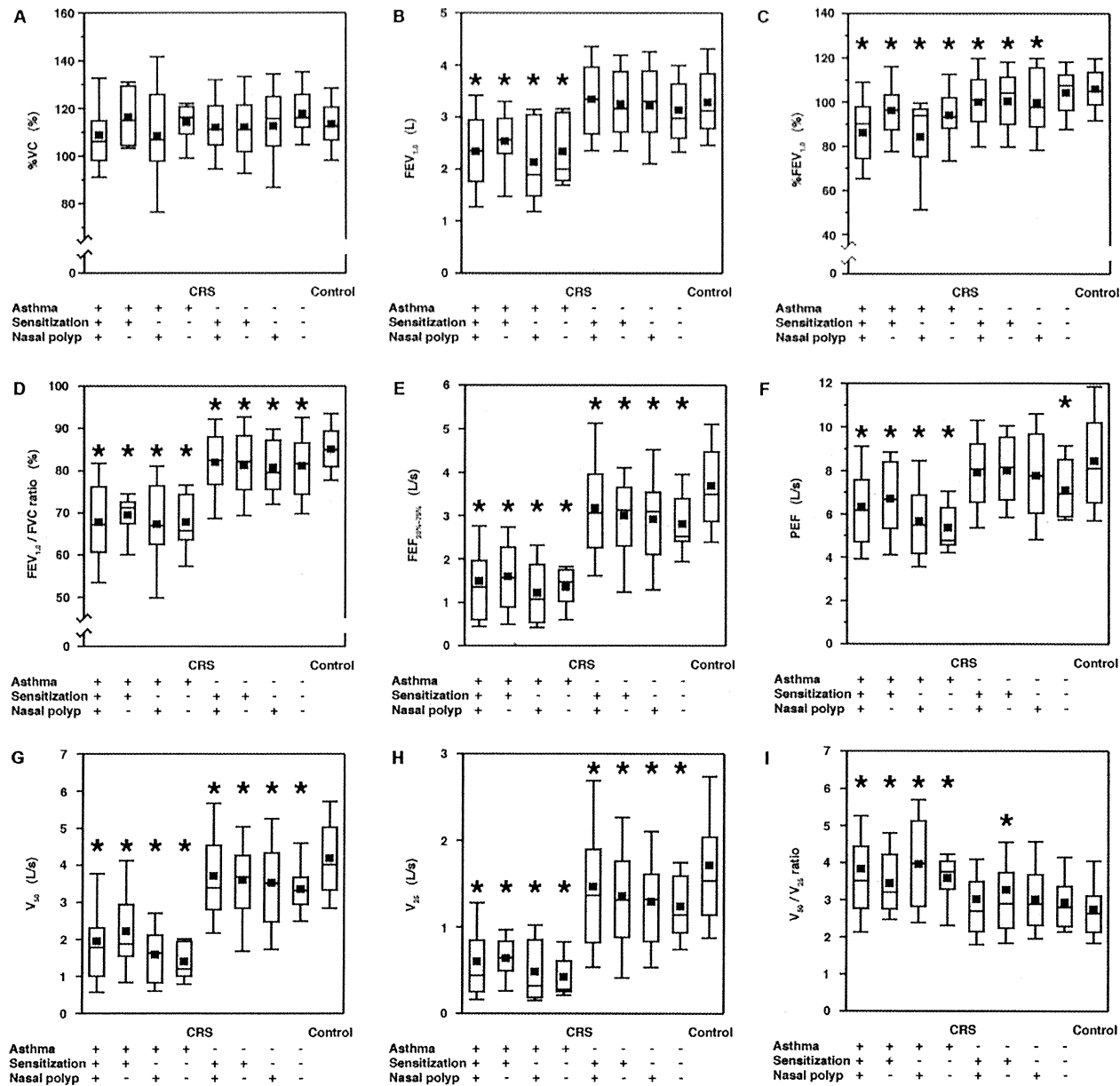


FIGURE 1. Pulmonary function in patients with chronic rhinosinusitis (CRS). (A) %VC. (B) FEV_{1.0}. (C) Percent predicted FEV_{1.0}. (D) FEV_{1.0}/FVC ratio. (E) FEF_{25%-75%}. (F) PEF. (G) V₅₀. (H) V₂₅. (I) V₅₀/V₂₅ ratio. The rectangle includes the range from the 25th to the 75th percentiles, the horizontal line indicates the median, and the vertical line indicates the range from the 10th to the 90th percentiles. The black square shows the mean value. *Indicates significant difference compared with normal controls. %VC = percent predicted vital capacity; FEF_{25%-75%} = mean forced expiratory flow between 25% and 75% of the forced vital capacity; FEV_{1.0} = forced expiratory volume in 1 second; FVC = forced vital capacity; PEF = peak expiratory flow; V₂₅ = maximal expiratory flow rate at 25% of vital capacity; V₅₀ = maximal expiratory flow rate at 50% of vital capacity.

TABLE 3. Clinical parameters of patients and normal controls

| | Chronic rhinosinusitis with asthma | | | | Chronic rhinosinusitis without asthma | | | | | |
|------------------------------------|------------------------------------|---------------|---------------|---------------|---------------------------------------|---------------|---------------|---------------|--------------|---------|
| Sensitization to inhaled allergens | | | | | | | | | | |
| | Positive | | Negative | | Positive | | Negative | | | |
| Nasal polyposis | Present | Absent | Present | Absent | Present | Absent | Present | Absent | Control | p |
| Serum total IgE levels (IU/mL) | 445.9 ± 498.8 | 352.0 ± 658.7 | 114.6 ± 93.3 | 242.0 ± 183.1 | 362.6 ± 521.7 | 462.0 ± 547.1 | 60.1 ± 62.3 | 62.7 ± 83.0 | N/A | 0.001* |
| Peripheral blood eosinophil count | 749.1 ± 433.8 | 328.6 ± 175.3 | 585.2 ± 310.7 | 571.1 ± 473.7 | 391.1 ± 335.8 | 206.5 ± 168.2 | 214.6 ± 174.0 | 182.7 ± 146.9 | 165.4 ± 75.6 | <0.001* |
| Lund-Mackay CT score | 18.5 ± 4.8 | 6.0 ± 4.7 | 18.4 ± 5.1 | 13.6 ± 8.1 | 11.5 ± 5.5 | 6.8 ± 4.5 | 12.2 ± 6.5 | 6.4 ± 3.9 | N/A | <0.001* |

Data represent means ± standard deviation.
*One-way analysis of variance.
CT = computed tomography; IgE = immunoglobulin E; N/A = not applicable.

Epidemiological and pathophysiological connections between CRS and lower lung diseases have been suggested.^{11–14} Allergic rhinitis and its impact on asthma has been thoroughly studied; however, the relationship between nonallergic sinus disease and asthma is not yet fully understood.^{4,15} Some clinical studies have shown that the treatment of CRS, including medical or endoscopic sinus surgery, demonstrated a beneficial effect on the symptoms of concomitant asthma.^{16–19} This study revealed that CRS patients had affected lung function regardless of asthma. Although the subjects in this study were not clinically diagnosed as having COPD, CRS patients without asthma had latent obstructive lung function.

Total serum IgE levels and peripheral blood eosinophil counts are widely examined, and are remarkable biomarkers for evaluating patients with asthma.²⁰ This study showed that nonasthmatic CRS patients with sensitization and asthmatic patients had a statistically increased eosinophil count and high serum IgE level. These findings suggest that similar inflammatory processes are involved in both CRS with allergic sensitization and asthma. In addition, we also found that nonasthmatic CRS patients without sensitization did not show increased total serum IgE levels and peripheral blood eosinophil counts, but had latent obstructive lung function. These findings suggest that nonallergic sinus disease might be related to obstructive lower respiratory disease.

The Lund-Mackay CT score is widely used for assessing the disease severity of CRS.⁸ Mehta et al.²¹ reported that blood and sputum eosinophil levels in patients with asthma are directly correlated with sinus mucosal thickening assessed by Lund-Mackay CT score. In the present study, we observed that CRS patients with nasal polyposis had a higher Lund-Mackay CT score than ggCRS patients without nasal polyposis.

Various explanations for the upper and lower airway association in the pathogenesis of united airway diseases have been presented. These hypotheses include the 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.^{4,15,22,23} Clinical parameters in this study (IgE levels, peripheral blood eosinophil counts, and CT score) did not significantly correlate with the spirometric parameters. A recent study showed that inflammatory cytokines in nasal secretion might be associated with decreased lung function.²⁴

The extent of the impact of upper respiratory disease on lower respiratory function is currently under debate. Dixon et al.²⁵ reported that asthmatics without rhinitis tend to have lower lung function than asthmatics with rhinitis. In contrast, another report showed that allergic rhinitis and sinusitis were associated with more severe asthmatic symptoms.²⁶ The present findings clearly show that CRS patients had affected lung function, even in patients who were not clinically diagnosed as having lower lung disease. Although the strong relationship between allergic rhinitis and asthma is well established, the effect of CRS on the pathogenesis of lower lung diseases remains controversial. Further investigation into the causes of loss of lung function in patients with CRS is required to clarify the relationship between upper and lower respiratory tract disorders.

Conclusion

This study showed that pulmonary function was affected in patients with CRS, regardless of sensitization status. The CRS patients without asthma or COPD had latent obstructive lung function changes. Clinicians should be aware of the condition of the lower respiratory tract in patients with CRS.

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Staphylococcal protein A–formulated immune complexes suppress enterotoxin-induced cellular responses in nasal polyps

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Background: Recent studies have revealed that *Staphylococcus aureus* and its components participate in the pathogenesis of eosinophilic airway diseases, such as chronic rhinosinusitis with nasal polyps.

Objective: We sought to determine whether staphylococcal protein A (SpA) from *S aureus* regulated cellular responses in nasal polyps, especially when coupled to immunoglobulins in immune complexes (ICs).

Methods: Dispersed nasal polyp cells (DNPCs) or peripheral blood monocytes were cultured *in vitro* with SpA in the presence or absence of IgG, and IL-5, IL-13, IFN- γ , IL-17A, and IL-10 levels were measured in the supernatants. The effect of SpA exposure on staphylococcal enterotoxin B–induced cytokine production by DNPCs in the presence and absence of IgG, IgA, and autologous serum was also examined.

Results: Exposure to SpA induced DNPCs to produce significantly higher IL-10, IL-13, and IL-17A levels than DNPCs without SpA, although the magnitude of the IL-17A increase was less than that of IL-10 and IL-13. SpA induced IL-10 production mainly from adherent DNPCs, and this was significantly enhanced in the presence of IgG; similar results were observed in peripheral blood monocytes. IC formation between SpA and IgG (SpA-IgG ICs) was confirmed by using native polyacrylamide gel electrophoresis. SpA-IgG ICs, but not SpA alone, almost completely suppressed staphylococcal enterotoxin B–induced IL-5, IL-13, IFN- γ , and IL-17A production by DNPCs; similar inhibition was observed in DNPCs treated with SpA in the presence of either IgA or autologous serum.

Conclusions: Our results suggest that SpA can regulate the pathogenesis of enterotoxin-induced inflammation in patients with chronic rhinosinusitis with nasal polyps through coupling to immunoglobulins. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: Chronic rhinosinusitis with nasal polyps, cytokines, enterotoxins, immune complexes, immunoglobulins, immune evasion, *Staphylococcus aureus*, staphylococcal protein A

Microbes, including viruses, fungi, and bacteria, can elicit cellular responses in patients with chronic rhinosinusitis with nasal polyps (CRSwNP).^{1,2} *Staphylococcus aureus* is one of the most common bacterial species found in both mucin and the sinus epithelium in patients with chronic rhinosinusitis (CRS).² Of particular concern, *S aureus* releases a number of molecules associated with CRS pathogenesis, including exotoxins and biofilm components.²⁻⁶

S aureus plays several roles in airway inflammation.⁷⁻¹⁰ For example, *S aureus* stimulates release of the eosinophil chemoattractant eotaxin from human airway epithelial cells.⁷ In contrast, administration of formalin-fixed *S aureus* particles into mice followed by intranasal allergen challenge induces T_H1-biased immune responses and prevents airway inflammation, including local eosinophilia.⁹ In human subjects *S aureus* exposure induces secretion of the immunosuppressive cytokine IL-10 by monocytes, as well as by cocultured B cells and plasmacytoid dendritic cells.^{10,11}

Staphylococcal protein A (SpA) is a major surface protein on almost all *S aureus* strains, especially respiratory isolates, and is freely secreted into the extracellular environment.^{12,13} SpA can bind to various host-derived proteins, including the Fc and V_H3 domains of immunoglobulins, von Willebrand factor, complement C3, epidermal growth factor receptor, and TNF- α receptor 1 (TNFR1). Thus SpA can potentially modulate host immune responses to *S aureus* in various ways,¹³⁻¹⁵ as illustrated in the following examples. SpA-TNFR1 signaling can induce IL-8 expression in human airway epithelial cells through nuclear factor κ B activation.¹⁴ Intranasal exposure to SpA can lead to airway hyperresponsiveness and eosinophilic inflammation in SpA-immunized mice,¹⁶ and SpA in combination with α -toxin can loosen tight junctions between airway epithelial cells.¹⁷ SpA can also act as a superallergen to induce B-cell apoptosis.¹³ Moreover, binding of SpA to epidermal growth factor receptor or TNFR1 on epithelial cells and macrophages induces shedding of TNFR1, which can then neutralize available TNF- α .^{12,17,18} Thus depending on its binding partner and responding cell type in a host, SpA can act as either a proinflammatory or anti-inflammatory molecule.¹⁷

Despite the fact that SpA is detected in sinonasal tissues, especially in eosinophilic nasal polyps, little is known about how or even whether SpA regulates inflammation in patients with CRSwNP.¹⁹ Patou et al²⁰ reported that a 30-minute exposure to SpA led to histamine, leukotriene C₄/D₄/E₄, and prostaglandin

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Abbreviations used

| | |
|---------|---|
| CRS: | Chronic rhinosinusitis |
| CRSsNP: | Chronic rhinosinusitis without nasal polyps |
| CRSwNP: | Chronic rhinosinusitis with nasal polyps |
| DNPC: | Dispersed nasal polyp cell |
| IC: | Immune complex |
| SEB: | Staphylococcal enterotoxin B |
| SpA: | Staphylococcal protein A |
| SpA+AS: | Staphylococcal protein A mixed with 5% autologous serum |
| TLR: | Toll-like receptor |
| TNFR1: | TNF- α receptor 1 |

D₂ release by nasal polyp fragments.²⁰ In the present study we sought to determine the effect of SpA on proinflammatory and anti-inflammatory cytokine production by using an *ex vivo* CRSwNP model.⁶ Moreover, because SpA can strongly couple to immunoglobulins to form immune complexes (ICs) and the noses of patients with CRSwNP have high levels of immunoglobulins, such as IgG and IgA,²¹ we investigated the effect of SpA-containing ICs on the previously observed staphylococcal enterotoxin B (SEB)-induced cytokine production by nasal polyp cells.¹⁴

METHODS**Patients**

Nasal polyps were surgically excised from 52 Japanese patients with CRSwNP (age range, 17–82 years; mean age, 54.8 years). CRSwNP was diagnosed based on the criteria reported in the “European position paper on rhinosinusitis and nasal polyps 2012.”²² Exclusion criteria were described previously.⁶ Eleven patients (age range, 35–75 years; mean age, 55.4 years) with chronic rhinosinusitis without nasal polyps (CRSsNP) were enrolled as control subjects.²² Informed consent for participation in the study was obtained from each patient or from a parent of patients less than 20 years old. The study was approved by the Human Research Committee of the Okayama University Graduate School of Medicine and Dentistry.

Antigen and reagents

SpA, RPMI 1640, L-glutamine/penicillin/streptomycin solution, protease, collagenase, hyaluronidase, DNase I, and FCS were purchased from Sigma (St Louis, Mo). Human IgG and IgA were purchased from Jackson ImmunoResearch Laboratories (West Grove, Pa). Red blood cell lysis buffer was purchased from Roche (Indianapolis, Ind). SEB was purchased from Toxin Technology (Sarasota, Fla). Ficoll-Paque was purchased from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). Rabbit complement was purchased from Cedarlane Laboratories (Hornby, Ontario, Canada). Rat anti-human IL-10 mAb was purchased from LifeSpan BioSciences (Seattle, Wash). Rat IgG₁ was purchased from R&D Systems (Minneapolis, Minn).

Culturing dispersed nasal polyp cells and uncinate tissue cells with SpA

Dispersed nasal polyp cells (DNPCs) were prepared from nasal polyps by means of enzymatic digestion with protease, collagenase, hyaluronidase, and DNase, as previously described.⁶ The frequency of DNPCs expressing c-kit (8.5% \pm 5.3%), eosinophil cationic protein/eosinophil peroxidase (11.7% \pm 8.9%), CD79 α (8.9% \pm 8.2%), CD68 (8.5% \pm 6.8%), CD4 (7.8% \pm 11.1%), CD8 (10.9% \pm 10.5%), cytokeratin (15.5% \pm 6.7%), and vimentin (21.6% \pm 7.7%) indicated the presence of both stromal and immune cells, including mast cells, eosinophils, B cells, macrophages, CD4⁺ and CD8⁺ T cells, epithelial cells, and fibroblasts/vascular endothelial cells,

respectively.³ DNPCs (2×10^6 /mL) were stimulated with 0.1, 1.0, or 10 μ g/mL SpA and incubated at 37°C and 5% CO₂. Supernatants were collected after 24 and 72 hours and stored at –80°C for subsequent cytokine analysis. For preparing adherent and nonadherent cells, DNPCs (2×10^6 /mL) were rested in a culture bottle (Sumitomo Bakelite, Tokyo, Japan) in RPMI 1640 supplemented with 10% heat-inactivated FCS and L-glutamine/penicillin/streptomycin solution. After incubation at 37°C and 5% CO₂ for 120 minutes, nonadherent cells were removed, and adherent cells were collected with a cell scraper (Becton Dickinson Labware, Franklin Lakes, NJ). Cells were then cultured in the presence or absence of 10 μ g/mL SpA for 24 hours. Alternatively, DNPCs were cultured in the presence of human IgG (100 μ g/mL) with or without 10 μ g/mL SpA, and supernatant was collected 24 hours later. Dispersed uncinate tissue cells were prepared from the uncinate mucosa of patients with CRSsNP and cultured with or without 10 μ g/mL SpA; supernatant was collected 24 hours later.

Culturing human monocytes with SpA

Heparinized blood was collected from 8 healthy Japanese volunteers (age range, 26–48 years; mean age, 34.3 years), and PBMCs were isolated, as previously described.²³ Monocytes were generated from PBMCs by using a positive-selection method with the MACS Monocyte Isolation kit II (Miltenyi Biotec, Bergisch Gladbach, Germany) and cultured with 10 μ g/mL SpA in the presence or absence of 100 μ g/mL human IgG, and the supernatant was collected 24 hours later. In a separate experiment monocytes were cultured with a mixture of SpA and IgG in the presence or absence of complement (10% normal rabbit serum).

Effect of SpA and SpA-containing ICs on SEB-induced cytokine production by DNPCs

DNPCs were treated with 10 μ g/mL SpA in the presence or absence of 100 μ g/mL human IgG or IgA and then immediately stimulated with 1 ng/mL SEB for 72 hours. In some experiments anti-human IL-10 mAb or control rat IgG₁ (20 μ g/mL) was also added to the culture.

Effect of autologous serum on SpA-mediated regulation

Serum was collected from each patient immediately before surgery. Some of the autologous serum was heated for 30 minutes at 56°C with mixing to inactivate complement. DNPCs were stimulated with 10 μ g/mL SpA in the presence or absence of freshly isolated native autologous serum (0.2%, 1%, and 5%) for 24 hours. In some experiments DNPCs were first treated with a mixture of SpA and either native or heat-inactivated autologous serum and then stimulated with 1 ng/mL SEB for 72 hours.

Cytokine determination

IL-5, IL-13, IL-17A, IFN- γ , IL-10, and IL-12 p70 levels in culture supernatants were determined by means of ELISA.¹ IL-5, IFN- γ , and IL-10 levels were measured with Opt EIA sets (BD Biosciences, San Jose, Calif), according to the manufacturer's instructions. IL-12 p70 and IL-17A levels were measured by using a DuoSet ELISA development kit (R&D Systems). IL-13 levels were measured by using paired capture and detection antibodies (BD Biosciences) and recombinant standards (R&D Systems). The detection limit for each assay was 4 pg/mL for IL-5, 2 pg/mL for IL-12 p70, 2 pg/mL for IL-13, 8 pg/mL for IL-17A, 4 pg/mL for IFN- γ , and 8 pg/mL for IL-10.

Polyacrylamide gel electrophoresis

Native polyacrylamide gel electrophoresis (4–15% Mini-Protean TGX gels; Bio-Rad Laboratories, Hercules, Calif) was performed to confirm the formation of a complex between SpA and IgG.²⁴ SpA (1 μ g) and human IgG (11 μ g) in Tris-glycine buffer (pH 8.3) were loaded onto gel lanes alone or after being mixed together for various time periods (1 minutes, 30 minutes,

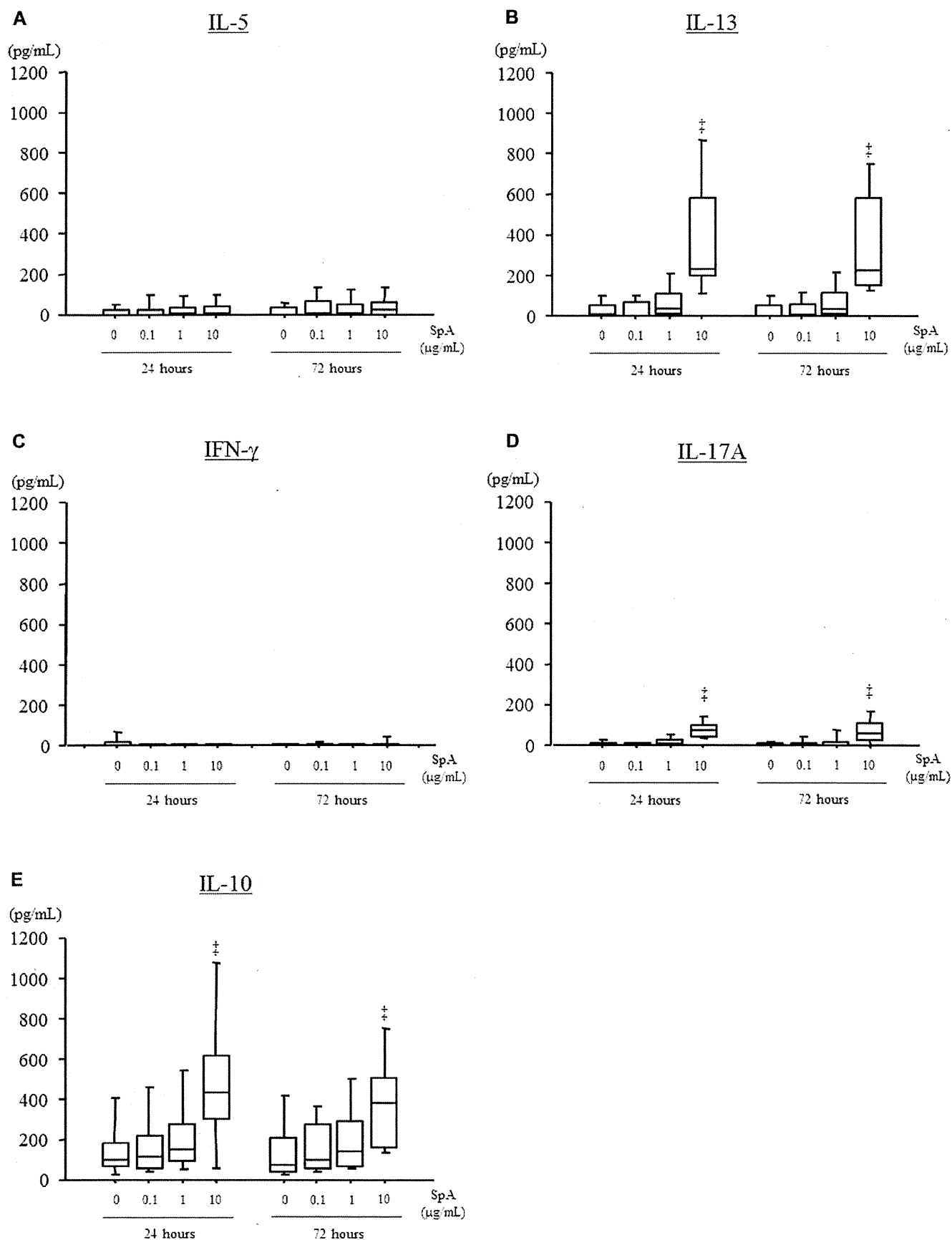


FIG 1. SpA-induced IL-5 (A), IL-13 (B), IFN- γ (C), IL-17A (D), and IL-10 (E) production by nasal polyp cells. Within the box plots, the box represents the 25th to 75th percentile range, the horizontal line indicates the median, and the vertical line indicates the 10th to 90th percentile range. *P* values were determined by using the Dunn test. $\ddagger P < .001$.

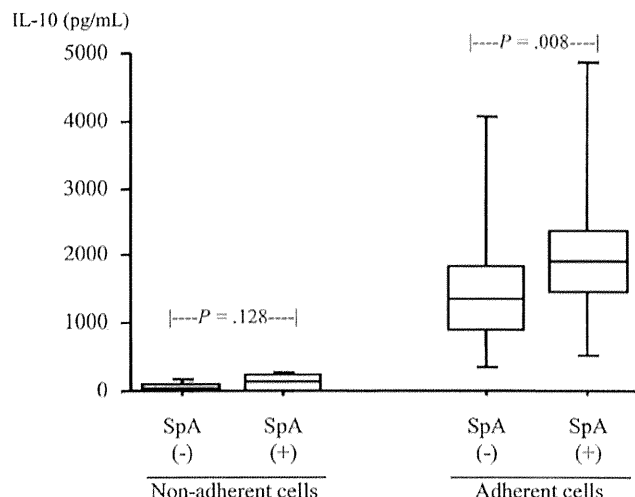


FIG 2. Cellular source of SpA-induced IL-10 production in nasal polyp cells. Nonadherent and adherent cells were cultured with or without 10 μ g/mL SpA for 24 hours, and IL-10 levels in the supernatants were measured. *P* values were determined by using the Wilcoxon signed-rank test.

1 hour, or 4 hours) and electrophoresed. Gels were stained with BioSafe Coomassie G250 to detect precipitated protein (Bio-Rad Laboratories).

Statistical analysis

Values are given as medians. The nonparametric Mann-Whitney *U* test was used to compare data between groups, and the Wilcoxon signed-rank test was used for analysis within groups. A Kruskal-Wallis test followed by a Dunn test was used for multiple comparisons. *P* values of less than .05 were considered statistically significant. Statistical analyses were performed with SPSS software (version 11.0; SPSS, Chicago, Ill).

RESULTS

SpA induces cytokine production by nasal polyp cells

Incubation of DNPCs with various SpA doses for 24 or 72 hours significantly increased IL-13 ($P < .001$ at 24 and 72 hours), IL-17A ($P < .001$ at 24 and 72 hours), and IL-10 ($P = .002$ at 24 and 72 hours) production in a dose-dependent manner but not IL-5 ($P = .879$ at 24 hours and $P = .647$ at 72 hours) or IFN- γ ($P = .400$ at 24 hours and $P = .238$ at 72 hours) production. A Dunn test further revealed that SpA significantly increased IL-13, IL-17, and IL-10 production from DNPCs at the 10 μ g/mL dose ($P < .001$, Fig 1). This significant increase in levels of the immunosuppressive IL-10 cytokine led us to focus on investigating the regulatory effects of SpA in this study, especially with respect to IL-10.

SpA induces IL-10 production primarily from adherent cells in nasal polyps and SpA-containing ICs enhance IL-10 production

Because a variety of cell types could produce IL-10, we first sought to determine which cell types produced IL-10 in response to SpA²⁵ by comparing the effect of SpA on nonadherent versus adherent DNPCs. No significant increase in IL-10 production was observed from nonadherent DNPCs in response to SpA ($n = 9$, $P = .128$). In contrast, adherent DNPCs spontaneously

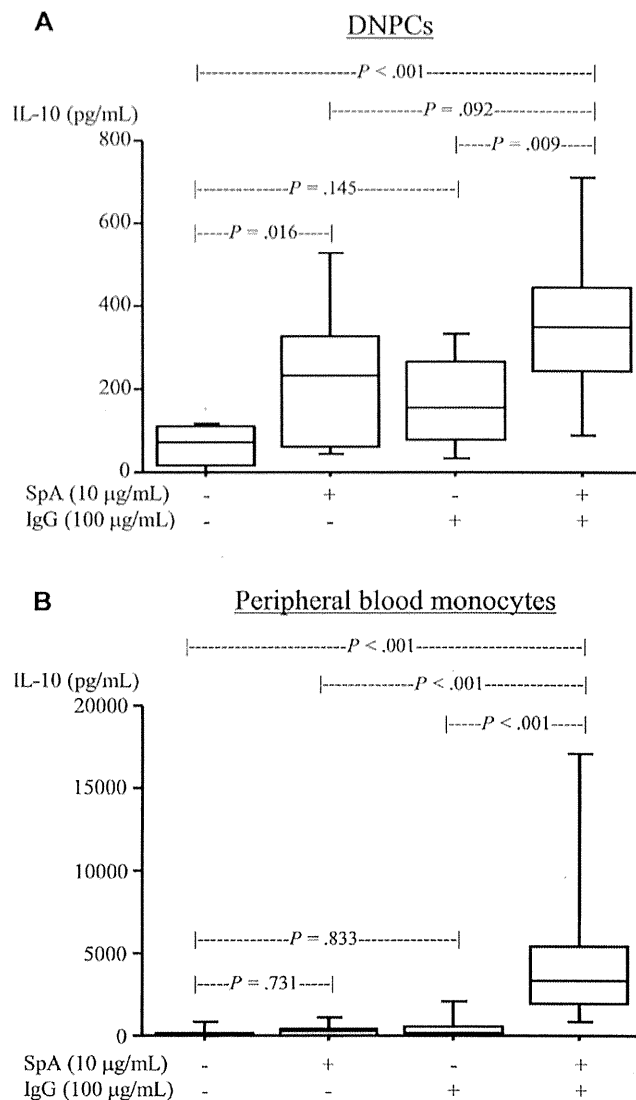


FIG 3. Enhanced IL-10 production by cells cultured with SpA plus IgG. DNPCs (A) and peripheral blood monocytes (B) were stimulated with SpA (10 μ g/mL) in the presence or absence of IgG (100 μ g/mL) for 24 hours, and IL-10 levels in the supernatants were measured. *P* values were determined by using the Dunn test.

produced higher IL-10 levels than nonadherent cells at baseline, and SpA significantly enhanced this IL-10 production ($P = .008$, Fig 2). This increase was not observed in SpA-treated dispersed uncinate tissue cells from control subjects with CRSsNP ($n = 11$, $P = .588$, see Fig E1 in this article's Online Repository at www.jacionline.org), suggesting that adherent cells in nasal polyps might be the primary source of SpA-induced IL-10 production in patients with CRS.

SpA can couple with immunoglobulins, especially IgG, to form ICs that affect immune responses.²⁶ To begin testing whether SpA-containing ICs altered DNPC responses, we first confirmed that SpA could form ICs within minutes of being mixed with human IgG (SpA-IgG ICs) under our culture conditions (see Fig E2 in this article's Online Repository at www.jacionline.org). The Kruskal-Wallis test revealed a significant difference in IL-10 production by DNPCs in the presence or absence of 10 μ g/mL SpA with or without 100 μ g/mL IgG ($n = 10$,

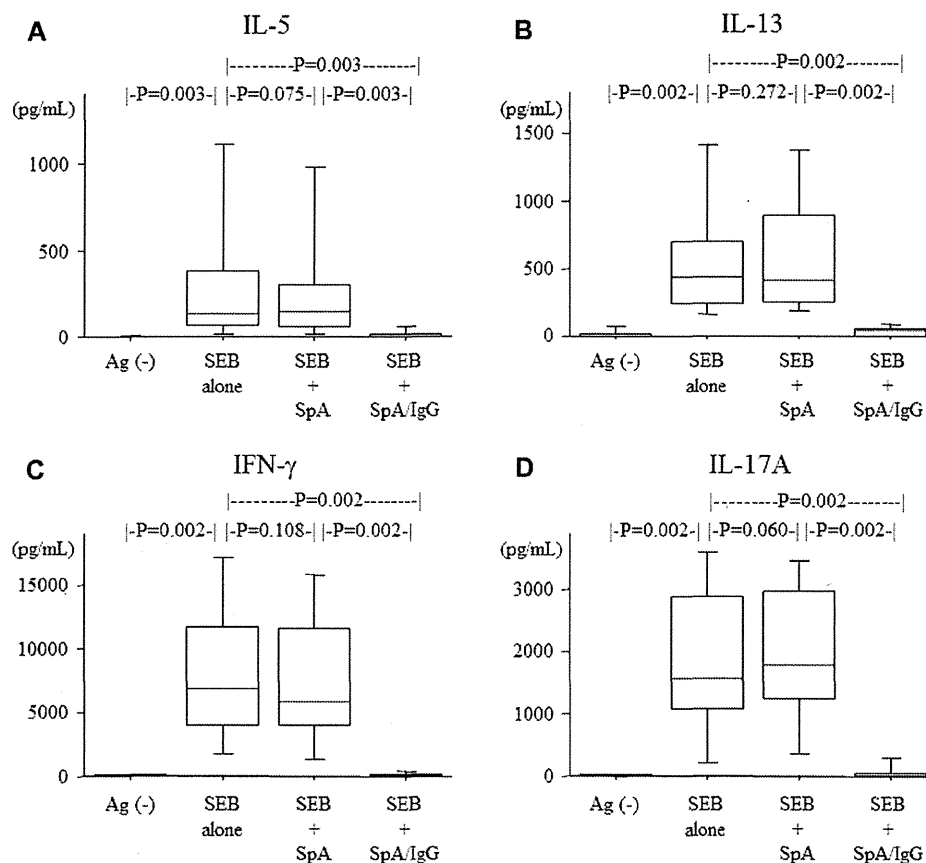


FIG 4. Effect of SpA-IgG ICs on SEB-induced IL-5 (A), IL-13 (B), IFN- γ (C), and IL-17A (D) production by nasal polyp cells. DNPCs were treated with SpA (10 μ g/mL) in the presence or absence of IgG (100 μ g/mL) and then stimulated with SEB (1 ng/mL) for 72 hours. *P* values were determined by using the Wilcoxon signed-rank test.

$P = .003$). Moreover, although the presence of 100 μ g/mL IgG itself did not significantly affect IL-10 production ($P = .145$), the addition of 10 μ g/mL SpA significantly enhanced IgG-induced IL-10 production from DNPCs ($P = .009$). A trend toward enhancing SpA-induced IL-10 production from DNPCs was observed in the presence of 100 μ g/mL IgG ($P = .092$, Dunn test; Fig 3, A), and statistical significance was achieved when confirming these results in human monocyte cultures ($n = 8$, $P < .001$; Fig 3, B). Although complement was previously shown to enhance IC-mediated cytokine production,^{27,28} the addition of complement (10% normal rabbit serum) did not alter IL-10 ($n = 6$, $P = .753$) or induce IL-12 production in IC-stimulated monocytes (see Fig E3 in this article's Online Repository at www.jacionline.org). We further tested the effect of ICs on adherent DNPCs alone and found that ICs induced significantly higher IL-10 levels than adherent DNPCs without ICs ($n = 8$, $P = .012$, see Fig E4 in this article's Online Repository at www.jacionline.org).

SpA-containing ICs functionally regulate enterotoxin-induced cellular responses by nasal polyp cells

Consistent with our previous study,⁶ exposure of DNPCs to SEB induced significantly higher IL-5 ($P = .003$), IL-13 ($P = .002$), IFN- γ ($P = .002$), and IL-17A ($P = .002$) levels

than untreated control cells (Fig 3). Therefore we next sought to determine whether SpA and/or SpA-containing ICs could exert a regulatory effect on this SEB-induced, eosinophilia-associated cytokine production by nasal polyp cells. Although no significant changes were observed in the presence of SpA alone ($P > .05$), SpA-IgG ICs dramatically suppressed SEB-induced IL-5 (95.5%, $P = .003$), IL-13 (94.0%, $P = .002$), IFN- γ (99.3%, $P = .002$), and IL-17A (97.8%, $P = .002$) production ($n = 12$; Fig 4). IL-10 played a role in this IC-mediated cytokine suppression because IL-10 blockade by anti-human IL-10 mAb (20 μ g/mL) partially yet significantly restored IL-5 ($P = .012$), IL-13 ($P = .018$), IFN- γ ($P = .028$), and IL-17A ($P = .028$) production compared with control rat IgG₁ ($n = 8$, see Fig E5 in this article's Online Repository at www.jacionline.org). With the exception of IL-13 ($P = .790$), similar inhibition was observed when SpA was mixed with IgA, although to a lesser extent than IgG ($n = 11$; see Fig E6 in this article's Online Repository at www.jacionline.org).

SpA mixed with autologous serum inhibits superantigen-induced cellular responses by nasal polyp cells

With respect to the clinical application of SpA-containing ICs to locally treat patients with CRSwNP, autologous serum would be a stronger candidate than allogeneic IgG in terms of lower

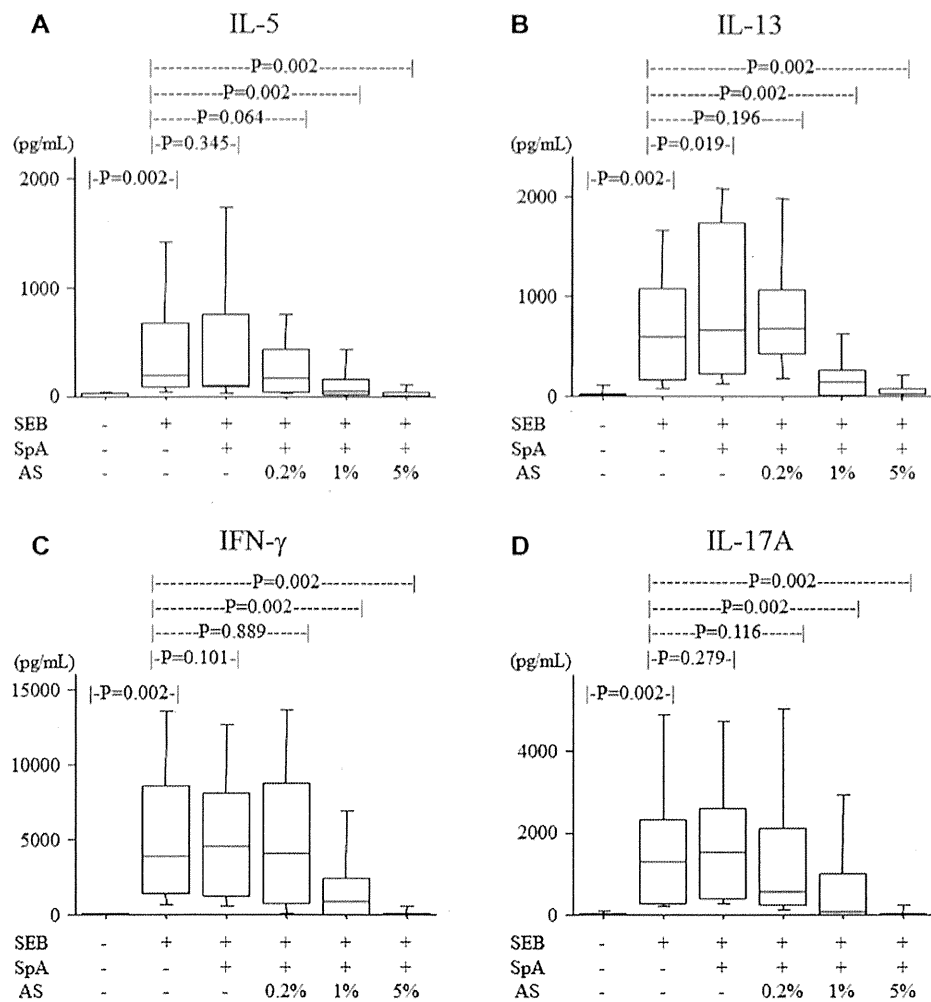


FIG 5. Effect of SpA plus autologous serum on SEB-induced IL-5 (A), IL-13 (B), IFN-γ (C), and IL-17A (D) production by nasal polyp cells. DNPCs were treated with SpA (10 μg/mL) in the presence of 0%, 0.2%, 1%, or 5% autologous serum (AS) and then stimulated with SEB (1 ng/mL) for 72 hours. P values were determined by using the Wilcoxon signed-rank test.

costs and risk of infection with unknown pathogens. Thus we next determined whether staphylococcal protein A mixed with 5% autologous serum (SpA+AS) could inhibit SEB-induced cytokine production by nasal polyp cells. Similar to SpA-IgG ICs, SpA+AS dose-dependently induced IL-10, but not IL-12, production by DNPCs (n = 13; see Fig E7 in this article’s Online Repository at www.jacionline.org). Furthermore, SpA+AS significantly suppressed SEB-induced IL-5 (92.0%, P = .002), IL-13 (93.7%, P = .002), IFN-γ (99.9%, P = .002), and IL-17A (99.9%, P = .002) production in a dose-dependent manner (n = 13; Fig 5); similar results were observed by using SpA mixed with heat-inactivated autologous serum (see Fig E8 in this article’s Online Repository at www.jacionline.org), indicating minimal involvement of complement in this suppression.

DISCUSSION

The present study reveals that SpA has both inflammatory and regulatory effects on the pathogenesis of CRSwNP. In particular, SpA-containing ICs strongly inhibited nasal polyp cell responses to SEB. Because *S aureus* freely secretes SpA into

the extracellular environment and immunoglobulins reside in the airways, these results might not only provide a basis for using SpA to treat airway inflammation but also enhance our current understanding of the mechanisms used by commensal bacteria, such as *S aureus*, to evade immune responses in the nose.^{18,24,26} Because our study is the first to report that SpA induces IL-10 production in the airways, we have focused on exploring the regulatory effect of SpA on CRSwNP. IL-10 produced in the respiratory tract limits inflammation in response to pathogens and allergens.^{29,30} Our results demonstrate that adherent nasal polyp cells are the primary source of SpA-induced IL-10 production. Together with the observed significant increase in IL-10 from peripheral blood monocytes after SpA exposure, these results suggest that SpA stimulates phagocytes (eg, macrophages) to produce IL-10. Parcina et al¹¹ recently demonstrated that *S aureus*-induced IL-10 release by cocultured B cells and plasmacytoid dendritic cells was impaired after simulation with an SpA-deficient strain of *S aureus*. Thus our results underscore the importance of SpA expression by *S aureus* for IL-10 production in human subjects. However, in the present study we observed that DNPC reactivity to SpA was highly variable among

the tested samples. Because DNPCs are bulk cell lines containing a wide variety of cells that vary in composition from sample to sample, differing proportions of adherent cells, including CD68⁺ macrophages, might lead to the observed variation in SpA-induced IL-10.³

The presence of IgG (or IgA) enhanced SpA-induced IL-10 production from both DNPCs and peripheral blood monocytes in our study. This result suggests that SpA-containing ICs are strong IL-10 inducers. Although ICs in combination with Toll-like receptor (TLR) ligands, such as LPS, can induce IL-10 production from myeloid cells, whether ICs alone can induce IL-10 remains a matter of debate.^{27,31,32} Insoluble ICs induce IL-10 production in human monocytes, but soluble ICs formed by heat-aggregated gamma globulins do not.^{27,32} IC-induced IL-10 production by myeloid cells is regulated by several factors, including the antigen/antibody ratio and IgG density within ICs.^{33,34} By using the *Limulus* ameobocyte lysate assay (Cape Cod, East Falmouth, Mass), we confirmed that endotoxin contamination in our SpA and IgG preparations was negligible (data not shown). Thus the observed induction of IL-10 by SpA-containing ICs might have been due to the rapid binding of SpA and IgG shown in Fig E2.

SpA-IgG ICs, but not SpA alone, dramatically inhibited SEB-induced IL-5, IL-13, IFN- γ , and IL-17A production by nasal polyp cells, suggesting that SpA-containing ICs are capable of both inducing IL-10 production and suppressing inflammation in patients with CRSwNP. ICs have anti-inflammatory properties due in part to their ability to enhance the generation of regulatory macrophages.^{29,31} MacLellan et al²⁶ recently demonstrated that intraperitoneally injecting 100 μ g of SpA every other day into mice substantially alleviated the clinical severity and histologic inflammation of collagen-induced arthritis. They also showed that SpA-IgG ICs significantly inhibited LPS-induced IL-12 and TNF- α production by human blood monocyte-derived macrophages. However, they did not detect any IL-10 production in their cultures. The discrepancy in IC-induced IL-10 production between their study and ours might be due to the different cell types (monocytes vs macrophages), concomitant TLR stimulation (with vs without LPS), or both. Our results suggest that SpA-IgG ICs have a broader anti-inflammatory effect involving T_H1-, T_H2-, and T_H17-type responses in patients with CRSwNP than in those with arthritis. In addition, blocking IL-10 partially abrogated the effect of SpA-IgG ICs on SEB-induced cytokine production, suggesting that ICs might also induce anti-inflammatory factors other than IL-10.

Although nearly 100% of clinical *S aureus* isolates harbor at least 1 superantigenic toxin, *S aureus* is one of the most common commensal bacterial species residing in the human upper respiratory tract.³⁵ The mechanism underlying immune evasion by *S aureus* in the nose is not fully understood. However, IL-10 production by nasal macrophages in response to TLR2 ligands (eg, peptidoglycans on the cell wall of *S aureus*) might play some role³⁵ because these ligands can downregulate superantigen-induced T-cell activation.³⁶ The results presented here are consistent with these reports in terms of finding that adherent DNPCs produce IL-10 and that IL-10 might play a role in SpA-mediated suppression of superantigen-induced cytokine production. Collectively, these findings suggest that both TLR2 ligands and SpA secreted by *S aureus* facilitate immune evasion by this organism in the nose by limiting superantigen-mediated inflammatory responses.

Autologous serum usually lacks antigenicity and contains a variety of essential components, including growth factors, immunoglobulins, and anti-inflammatory factors (eg, IL-1Ra and IL-10), that accelerate wound healing and tissue repair.^{37,38}

Indeed, topical application of autologous serum has been used to treat various types of wounds and inflammatory diseases, such as dry eye syndrome, tympanic membrane perforation, and osteoarthritis, without serious complications (eg, infections).³⁷⁻³⁹ Because *S aureus* frequently colonizes the human nose, our results demonstrating that a mixture of SpA and native autologous serum significantly suppresses SEB-induced cytokine production by DNPCs (>90% inhibition) suggest that autologous serum nasal drops might be a viable option for managing inflammatory sinonasal diseases. An SpA-containing silica column (Prosorba column; Fresenius HemoCare, Inc, Redmond, Wash) is currently used as an IgG immunoadsorption strategy for treating rheumatoid arthritis. Although low SpA levels have been detected in treated patients, indicating that this column can release SpA into the bloodstream, several clinical trials show that this technique does not induce serious side effects.⁴⁰ Thus we think that the potential risks of using intranasal autologous serum with SpA as a therapeutic approach to treat CRSwNP are low and that such treatment would pose a negligible risk to the patient.

Complement can regulate IC-stimulated IL-10 and IL-12 production by human immune cells, including monocytes and PBMCs.^{27,28} Our results using heat-inactivated serum suggest that complement has a minimal effect on amplifying inflammation rather than on IL-10 production for suppressing T_H2/T_H17-mediated inflammation in this model.

The SpA levels observed in the present study were similar to those shown in previous *in vitro* or *ex vivo* studies, which ranged from 8.4 to 200 μ g/mL.^{12,13,20} It would be helpful to know how these observed SpA levels correlate with the physiologic levels that occur during *Staphylococcus* species colonization and infection. Determining SpA concentrations together with exotoxin levels in nasal secretions should be investigated in future studies along with determining how the balance between SpA and exotoxins is altered during homeostatic and disease conditions.

In conclusion, we show evidence that SpA secreted by *S aureus* plays various roles in CRSwNP pathogenesis. In particular, SpA coupled with immunoglobulins in ICs strongly regulates eosinophilia-associated cytokine production from SEB-stimulated nasal polyp cells. Thus the present observations might provide a basis for developing novel therapeutic approaches that target SpA to manage airway inflammatory diseases. Because no animal models are currently available to investigate treating inflammatory diseases with SpA-formulated ICs, animal studies should be performed in the future to provide evidence to support such a therapeutic approach in human subjects. In addition, although SpA-containing ICs formed by using IgG or autologous serum strongly and broadly inhibited enterotoxin-induced T_H1, T_H2, and T_H17 inflammatory cytokine production, the possibility that improved bacterial survival by IL-10 induction could also drive further inflammation should be investigated.

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Clinical implications: Our findings might provide the basis for novel therapeutic approaches using SpA-containing ICs in managing eosinophilic airway diseases, including CRSwNP, allergic rhinitis, and asthma.

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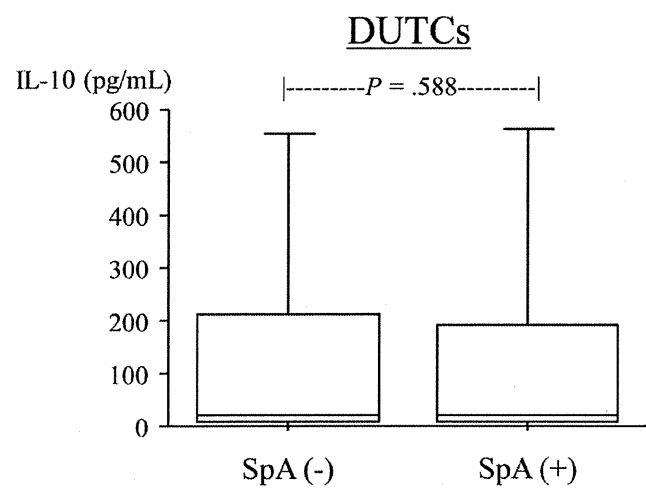


FIG E1. IL-10 production by dispersed uncinat tissue cells (*DUTCs*). Cells were stimulated with or without SpA (10 μ g/mL) for 24 hours, and IL-10 levels in the supernatant were measured. *P* values were determined by using the Wilcoxon signed-rank test.

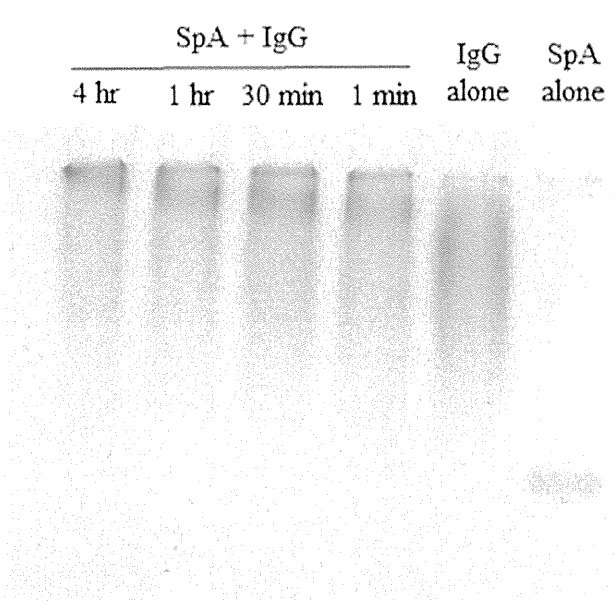


FIG E2. Formation of SpA and IgG ICs. SpA (1 μ g) and human IgG (11 μ g) were mixed for 1 minute, 30 minutes, 1 hour, or 4 hours and then subjected to 4–15% polyacrylamide gel electrophoresis under native conditions. After electrophoresis, the gel was stained with Coomassie brilliant blue. Lanes containing only SpA and IgG are also shown as controls.

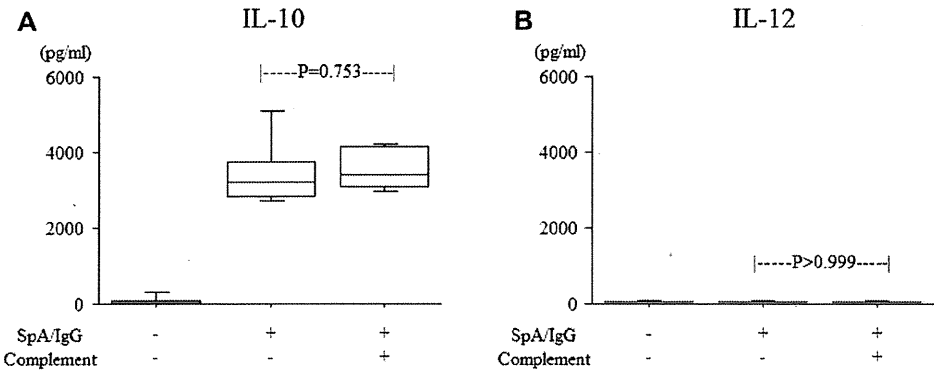


FIG E3. Effect of complement on SpA-IgG IC-induced IL-10 and IL-12 production by monocytes. Monocytes were cultured with the SpA and IgG mixture in the presence or absence of complement for 24 hours, and IL-10 (**A**) and IL-12 (**B**) levels in the supernatant were measured. *P* values were determined by using the Wilcoxon signed-rank test.

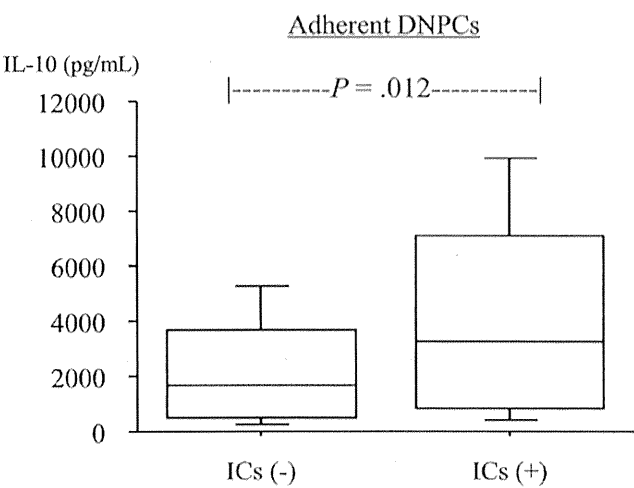


FIG E4. Effect of SpA-IgG ICs on IL-10 production by adherent cells in nasal polyps. Adherent DNPCs were cultured with or without the mixture of SpA (10 μ g/mL) and IgG (100 μ g/mL) for 24 hours, and IL-10 levels in the supernatant were measured. *P* values were determined by using the Wilcoxon signed-rank test.