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

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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, im-

balances 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 alphatoxin, *hla*. Also known as alpha-hemolysin, alphatoxin 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,
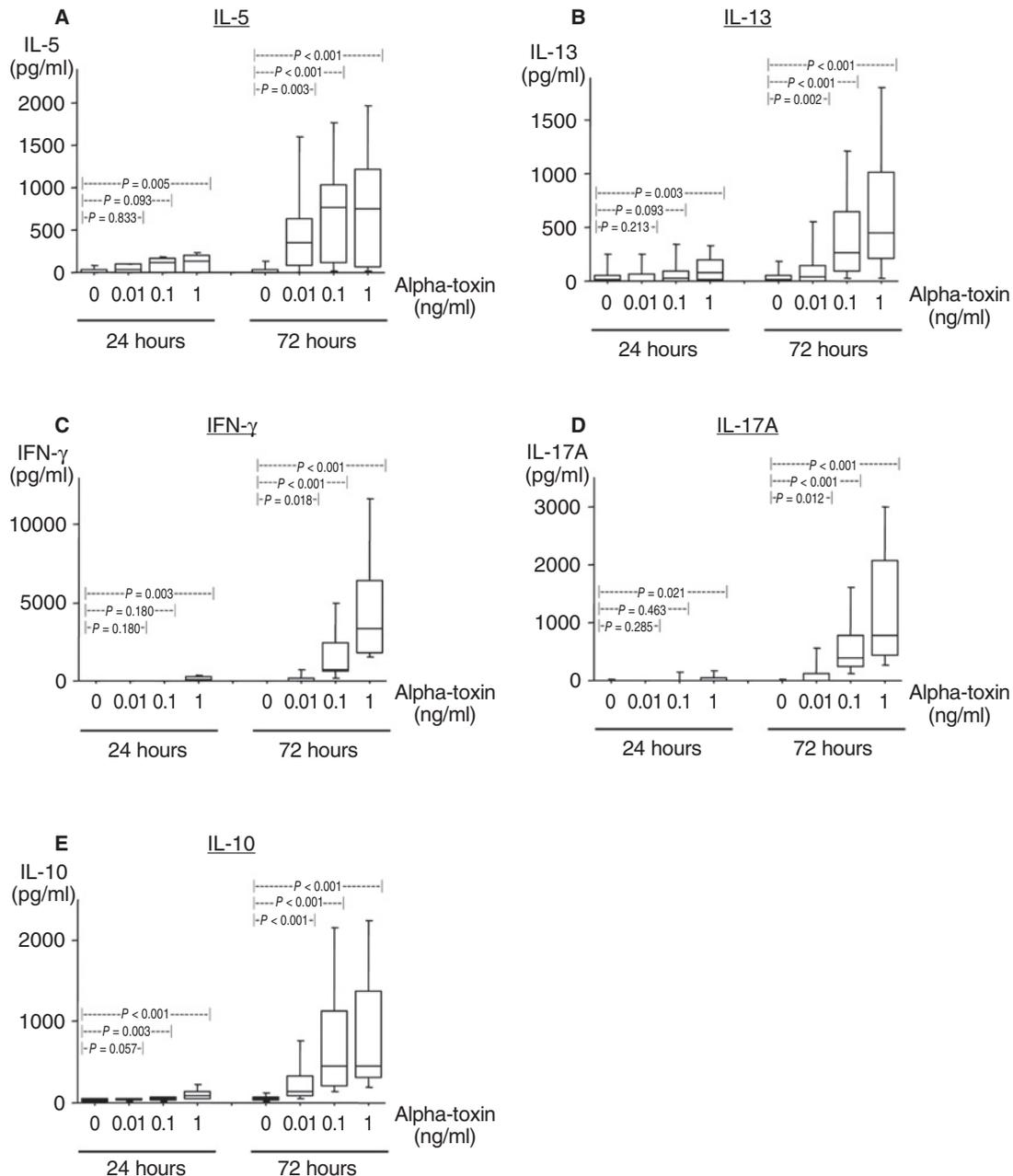
Fig. 1 Effect of alpha-toxin of *S. aureus* on IL-5 (**A**), IL-13 (**B**), IFN-γ (**C**), IL-17A (**D**) and IL-10 (**E**) production by DNPCs (*n* = 22). DNPCs were stimulated with 0.01, 0.1, or 1.0 ng/mL of alpha-toxin and incubated for either 24 or 72 hours. The rectangle includes the range from 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
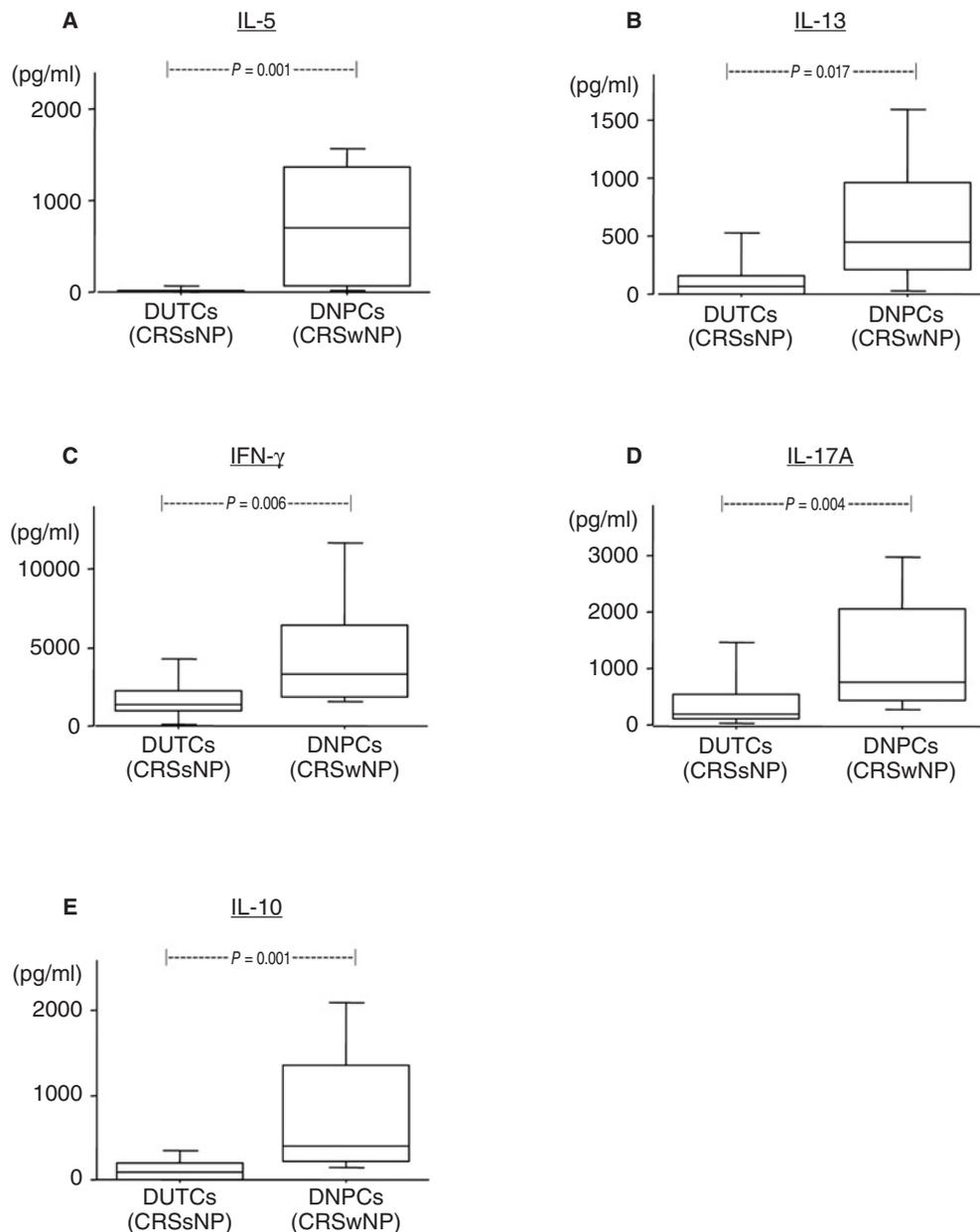
Fig. 2 Comparison of alpha-toxin-induced IL-5 (**A**), IL-13 (**B**), IFN-γ (**C**), IL-17A (**D**) and IL-10 (**E**) production between DNPCs from patients with CRSwNP (*n* = 22) and DUTCs from patients with CRSsNP (*n* = 9). The rectangle includes the range from the 25th to the 75th percentiles, the 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.
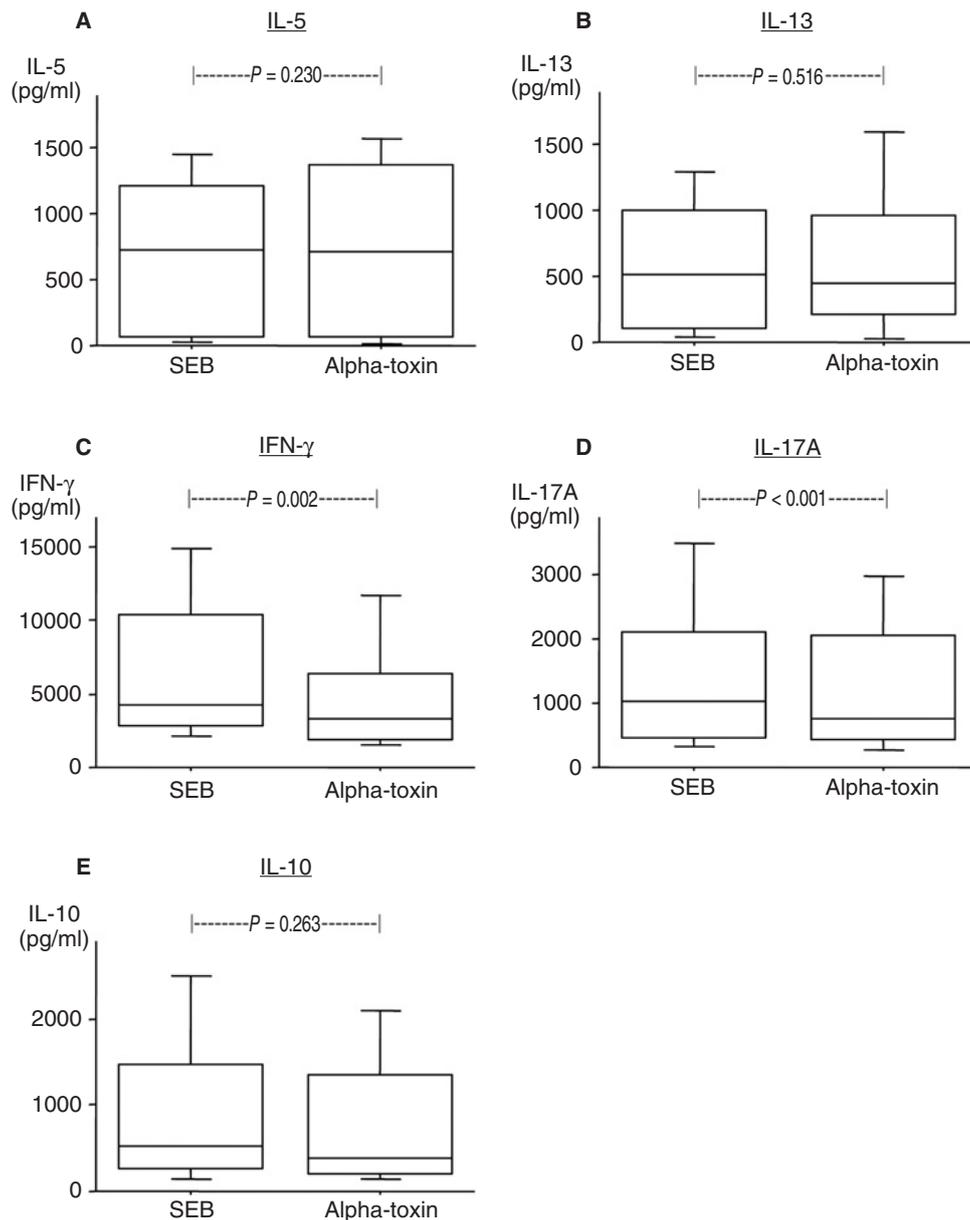
Fig. 3 Comparison of IL-5 (**A**), IL-13 (**B**), IFN-γ (**C**), IL-17A (**D**) and IL-10 (**E**) production by DNPCs between alpha-toxin and SEB stimulation. DNPCs ($n = 22$) were stimulated with 1.0 ng/mL of either alpha-toxin or SEB, and incubated for 72 hours. Bar indicates the median. The rectangle 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 (ρ = 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
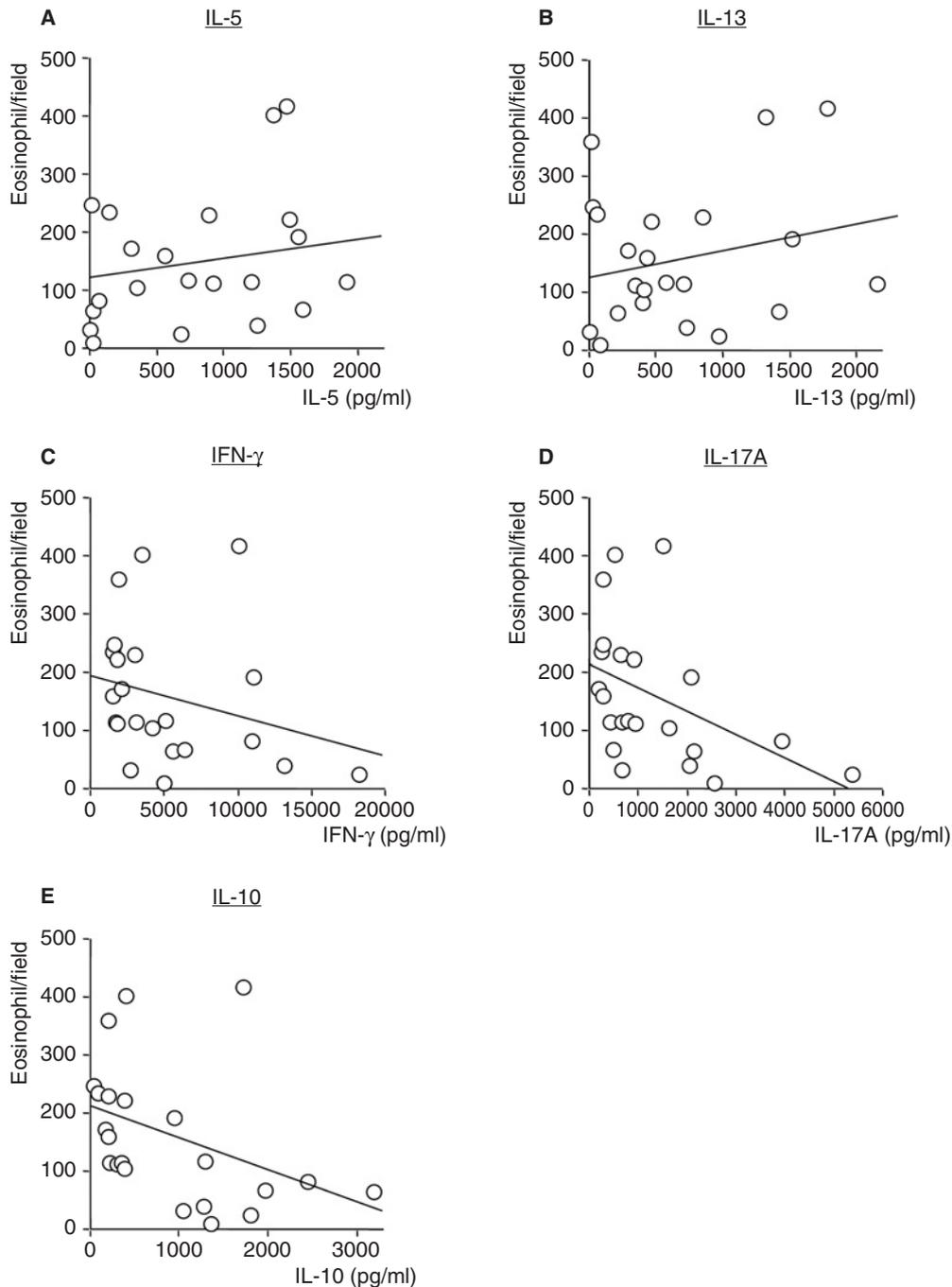
Fig. 4 Relationship between the number of eosinophils in nasal polyps and alpha-toxin-induced IL-5 (**A**), IL-13 (**B**), IFN-γ (**C**), IL-17A (**D**) and IL-10 (**E**) production by DNPCs (n = 22).

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 +/- stan-

dard 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
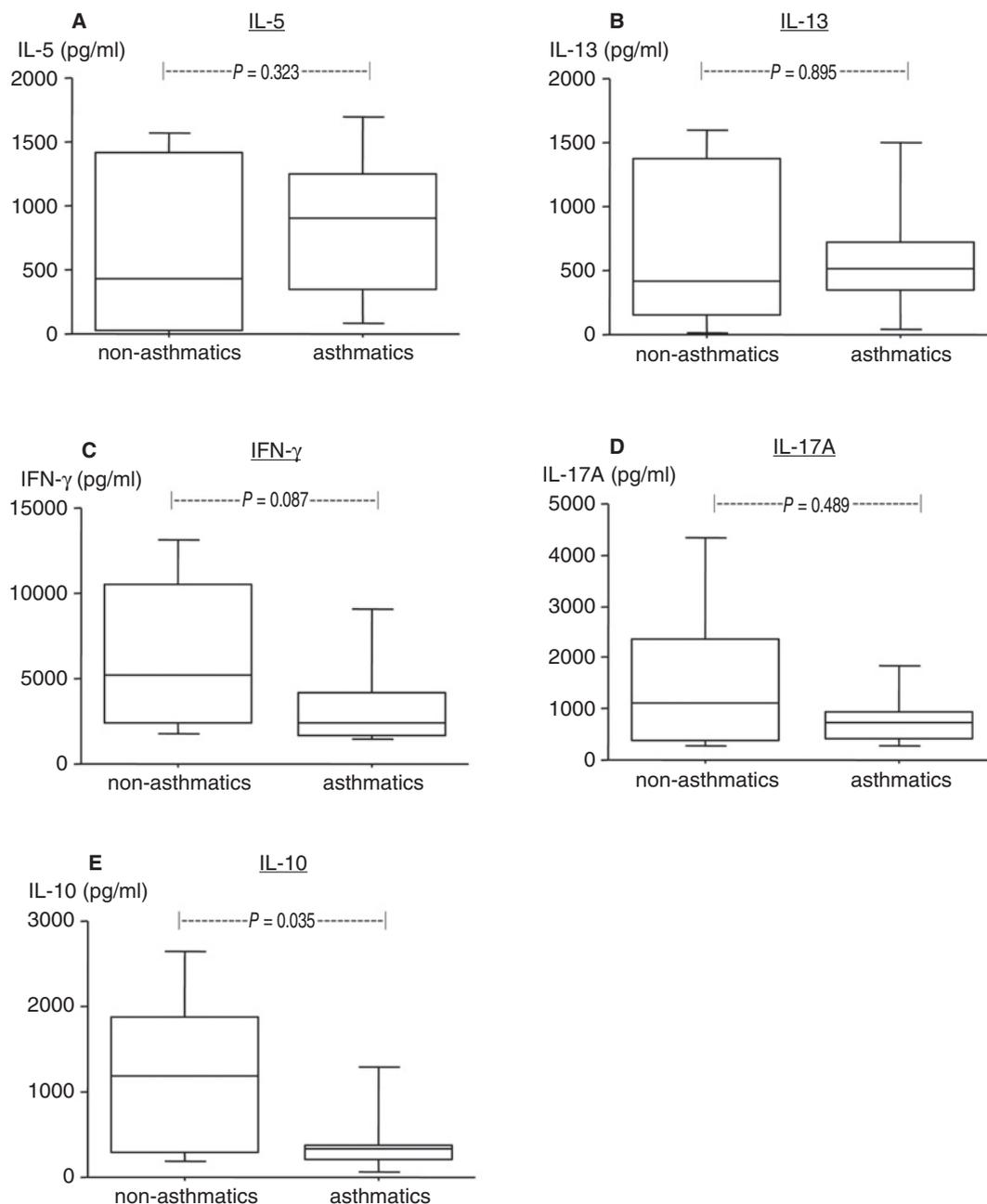
Fig. 5 Comparison of alpha-toxin-induced IL-5 (**A**), IL-13 (**B**), IFN-γ (**C**), IL-17A (**D**) and IL-10 (**E**) production by DNPCs between non-asthmatic ($n = 11$) and asthmatic ($n = 10$) patients. The rectangle includes the range from the 25th to the 75th percentiles, the horizontal line indicates 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.

REFERENCES

1. Bousquet J, Bachert C, Canonica GW *et al*, and Extended Global Allergy and Asthma European Network, World Allergy Organization and Allergic Rhinitis and its Impact on Ashtma Study Group. Unmet needs in severe chronic upper airway disease (SCUAD). *J Allergy Clin Immunol* 2009;**124**:428-33.
2. Zhang N, Van Zele T, Perez-Novo C *et al*. Different types of T-effector cells orchestrate mucosal inflammation in chronic sinus disease. *J Allergy Clin Immunol* 2008;**122**:961-8.
3. Makihara S, Okano M, Fujiwara T *et al*. Regulation and characterization of IL-17A expression in chronic rhinosinusitis and its relationship with eosinophilic inflammation. *J Allergy Clin Immunol* 2010;**126**:397-400.
4. Patou J, Gevaert P, van Zele T, Holtappels G, van Cauwenberge P, Bachert C. Staphylocossus aureus enterotoxin B, protein, and lipoteichoic acid stimulations in nasal polyps. *J Allergy Clin Immunol* 2008;**121**:110-5.
5. Okano M, Fujiwara T, Haruna T *et al*. PGE$_2$ suppresses staphylococcal enterotoxin-induced eosinophilia-associated cellular responses dominantly via an EP2-mediated pathway in nasal polyps. *J Allergy Clin Immunol* 2009;**123**:868-74.
6. Wang JH, Kwon HJ, Jang YJ. Rhinovirus upregulates matrix metalloproteinase-2, matrix-metalloproteinase-9, and vascular endothelial growth factor expression in nasal polyp fibroblasts. *Laryngoscope* 2009;**119**:1834-8.
7. Okano M, Fujiwara T, Haruna T *et al*. Role of fungal antigens in eosinophilia-associated cellular responses in nasal polyps: comparison with enterotoxin. *Clin Exp Allergy* 2011;**41**:171-8.
8. Perez-Novo CA, Jedzejzak-Czechowicz M, Lewandowska-Polak A *et al*. T cell inflammatory response, Foxp3 and TNFRS18-L regulation of peripheral blood mononuclear cells from patients with nasal polyps-asthma after staphylococcal superantigen stimulation. *Clin Exp Allergy* 2010;**40**:1323-32.
9. Bernstein JM, Allen C, Rich G *et al*. Further observations on the role of Staphylococcus aureus exotoxins and IgE in the pathogenesis of nasal polyposis. *Laryngoscope* 2011;**121**:647-55.
10. Sachs E, von Eiff C, Stoll W, Becker K, Rudack C. Induction of CXC chemokines in A549 airway epithelial cells by trypsin and staphylococcal proteases – a possible role for neutrophilic inflammation in chronic rhinosinusitis. *Clin Exp Immunol* 2006;**144**:534-42.
11. Tuan A, Baba T, Chen X *et al*. *Staphylococcus aureus* membrane and diacylated lipopeptide induce thymic stromal lymphopoietin in keratinocytes through the Toll-like receptor 2-Toll-like receptor 6 pathway. *J Allergy Clin Immunol* 2010;**126**:985-93.
12. Escotte S, Al Alan D, Le Naour R, Puchelle E, Guenounou M, Gangloff SC. T cell chemotaxis and chemokine release after *Staphylococcus aureus* interaction with polarized airway epithelium. *Am J Respir Cell Mol Biol* 2006;**34**:348-54.
13. Dinges MM, Orwin PM, Schlievert PM. Exotoxins of *Staphylococcus aureus*. *Clin Microbiol Rev* 2000;**13**:16-34.
14. Bhakdi S, Tranum-Jensen J. Alpha-toxin of *Staphylococcus aureus*. *Microbiol Rev* 1991;**55**:733-51.
15. Dragneva Y, Anuradha CD, Valeva A, Hoffmann A, Bhakdi S, Husmann M. Subcytocidal attack by staphylococcal alpha-toxin activates NF-kappaB and induced interleukin-8 production. *Infect Immun* 2001;**69**:2630-5.
16. Breuer K, Wittmann M, Kempe K *et al*. Alpha-toxin is produced by skin colonizing *Staphylococcus aureus* and induces a T helper type 1 response in atopic dermatitis. *Clin Exp Allergy* 2005;**35**:1088-95.
17. Niebuhur M, Scharonow H, Gathmann M, Mamerow D, Werfel T. Staphylococcal exotoxins are strong inducers of IL-22: A potential role in atopic dermatitis. *J Allergy Clin Immunol* 2010;**126**:1176-83.
18. Neibuhur M, Gathmann M, Scharonow H *et al*. Staphylococcal alpha-toxin is a strong inducer of interleukin-17 in humans. *Infect Immun* 2011;**79**:1615-22.
19. Kasraie S, Niebuhr M, Kopfnagel V, Dittrich-Breiholz O, Kracht M, Werfel T. Macrophages from patients with atopic dermatitis show a reduced CXCL10 expression in response to staphylococcal α-toxin. *Allergy* 2012;**67**:41-9.
20. Van Zele T, Gevaert P, Watelet JB *et al*. Staphylococcus aureus colonization and IgE antibody formation to enterotoxins is increased in nasal polyposis. *J Allergy Clin Immunol* 2004;**114**:981-3.
21. Peres AG, Madrenas J. The broad landscape of immune interactions with *Staphylococcus aureus*: From commensalism to lethal infections. *Burns* 2013;**39**:380-8.
22. Fokkens WJ, Lund VJ, Mullo J *et al*. European position paper on rhinosinusitis and nasal polyps. *Rhinol Suppl* 2012;**23**:1-298.
23. Hammilos DL, Leung DY, Huston DP, Kamil A, Wood R, Hamid Q. GM-CSF, IL-5 and RANTES immunoreactivity and mRNA expression in chronic hyperplastic sinusitis with nasal polyposis (NP). *Clin Exp Allergy* 1998;**28**:1145-52.
24. Schmid-Grendelmeire P, Altzauer F, Fischer B *et al*. Eosinophils express functional IL-13 in eosinophilic inflammatory diseases. *J Immunol* 2002;**169**:1021-7.
25. Yamamoto H, Okamoto Y, Horiguchi S, Kunii N, Yonekura S, Nakayama T. Detection of natural killer T cells in the sinus mucosa from asthmatics with chronic sinusitis. *Allergy* 2007;**62**:1451-5.
26. Faith A, Singh N, Farooque S *et al*. T cells producing the anti-inflammatory cytokine IL-10 regulate allergen-specific Th2 responses in human airway. *Allergy* 2012;**67**:1007-13.
27. Bantel H, Sinha B, Domschke W, Peters G, Schulze-Osthoff K, Jänicke RU. α-toxin is a mediator of *Staphylococcus aureus*-induced cell death and activates caspase via the intrinsic death pathway independently of death receptor signaling. *J Cell Biol* 2001;**155**:637-48.
28. Higaki T, Okano M, Fujiwara T *et al*. COX/PGE$_2$ axis critically regulates effects of LPS on eosinophilia-associated cytokine production in nasal polyps. *Clin Exp Allergy* 2012;**42**:1217-26.
29. Bachert C, Gevaert P, van Cauwenberge P. *Staphylococcus aureus* enterotoxins: a key in airway disease? *Allergy* 2002;**57**:480-7.
30. Van Zele T, Claeys S, Gevaert P *et al*. Differentiation of chronic sinus diseases by measurement of inflammatory mediators. *Allergy* 2006;**61**:1280-9.

31. Nakagome K, Okunishi K, Imamura M *et al*. IFN-γ attenuates antigen-induced overall immune responses in the airway as a Th1-type immune regulatory cytokine. *J Immunol* 2009;**183**:209-20.
32. Mitchell C, Provost K, Niu N, Homer R, Cohn L. IFN-γ acts on the airway epithelium to inhibit local and systemic pathology in allergic airway disease. *J Immunol* 2011;**187**:3815-20.
33. Boguniewicz M, Martin RJ, Martin D *et al*. The effects of nebulized recombinant interferon-γ in asthmatic airways. *J Allergy Clin Immunol* 1995;**95**:133-5.
34. Soyke MB, Wawrzyniak P, Eiwegger T *et al*. Defective epithelial barrier in chronic rhinosinusitis: The regulation of tight junction by IFN-γ and IL-4. *J Allergy Clin Immunol* 2012;**130**:1087-96.
35. Garcia G, Taille C, Laveneziana P, Bourdin A, Chanez P, Humbert M. Anti-interleukin-5 therapy in severe asthma. *Eur Respir Rev* 2013;**22**:251-7.
36. Lloyd CM, Hawrylowicz CM. Regulatory T cells in asthma. *Immunity* 2009;**31**:438-49.
37. Faith A, Singh N, Chevreton E *et al*. Counter regulation of the high affinity IgE receptor, FcεRI, on human airway dendritic cells by IL-4 and IL-10. *Allergy* 2009;**64**:1602-7.
38. Hyun MH, Lee CH, Kang MH, Park BK, Lee YH. Interleukin-10 promoter gene polypmorphisms and susceptibility to asthma: a meta-analysis. *PLoS One* 2013;**8**: e53758.
39. Kandane-Rathnayake RK, Tang ML, Simpson JA *et al*. Adult serum cytokine concentration and the persistence of asthma. *Int Arch Allergy Immunol* 2013;**161**:342-50.
40. Ogawa Y, Duru EA, Ameredes BT. Role of IL-10 in the resolution of airway inflammation. *Curr Mol Med* 2008;**8**: 437-45.
41. Akdis CA, Bachert C, Cingi C *et al*. Endotypes and phenotypes of chronic rhinosinusitis: A PRACTALL document of the European Academy of Allergy and Clinical Immunology and the American Academy of Allergy, Asthma & Immunology. *J Allergy Clin Immunol* 2013;**131**:1479-90.
42. Ikeda K, Tanno N, Tamura G *et al*. Endoscopic sinus surgery improves pulmonary function in patients with asthma associated with chronic sinusitis. *Ann Otol Rhinol Laryngol* 1999;**108**:355-9.
43. Dejima K, Hama T, Miyazaki M *et al*. A clinical study of endoscopic sinus surgery for sinusitis in patients with bronchial asthma. *Int Arch Allergy Immunol* 2005;**138**:97-104.
44. Proimos E, Papadakis CE, Chimona TS, Kiagiadaki D, Ferekidis E, Yiotakis J. The effect of functional endoscopic sinus surgery on patients with asthma and CRS with nasal polyps. *Rhinology* 2010;**48**:331-8.