

## Original Article

# Association between impaired IL-10 production following exposure to *Staphylococcus aureus* enterotoxin B and disease severity in eosinophilic chronic rhinosinusitis



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## Abbreviations:

CRS, chronic rhinosinusitis; CRSsNP, chronic

rhinosinusitis without nasal polyps;

CRSwNP, chronic rhinosinusitis with nasal

polyps; ECRS, eosinophilic chronic

rhinosinusitis; FEV<sub>1</sub>/FVC, 1-s forced

expiratory volume/forced vital capacity;

JESREC, Japanese Epidemiological Survey of

Refractory Eosinophilic Chronic

Rhinosinusitis; NP, nasal polyps;

SEB, staphylococcal enterotoxin B;

UT, uncinate tissues

## ABSTRACT

**Background:** IL-10 is a major anti-inflammatory cytokine that prevents inflammation-mediated tissue damage. We characterized the production of IL-10 by sinonasal tissue cells following exposure to *Staphylococcus aureus* enterotoxin B (SEB), which elicits cellular responses and is associated with the pathogenesis of eosinophilic chronic rhinosinusitis (ECRS).

**Methods:** Dispersed nasal polyp (NP) cells and uncinate tissue (UT) cells were prepared from patients with CRS with and without NP, respectively. Cells were incubated with SEB, and then the levels of IL-10 in the cell supernatants were determined. The effect of neutralizing IL-10 on SEB-induced IL-5, IL-13, IFN- $\gamma$ , and IL-17A production was examined. Expression of IL-10 in NPs was also determined.

**Results:** IL-10 was expressed in infiltrating inflammatory cells in NPs. NP cells, especially non-adherent NP cells, produced substantial amounts of IL-10 in response to SEB. Although baseline production of IL-10 was significantly higher in NP cells than UT cells, the degree of IL-10 response to SEB was not significantly different between the cell types. The degree of IL-10 production was negatively correlated with the degree of eosinophilia both in tissues and peripheral blood whereas positively correlated with the 1-s forced expiratory volume/forced vital capacity ratio. Patients with severe ECRS displayed a significant decrease in IL-10 production compared with those with non-ECRS. IL-10 neutralization significantly augmented SEB-induced IL-13 and IFN- $\gamma$  production by NP cells.

**Conclusions:** Impaired IL-10 production in response to SEB in NP may exacerbate the pathophysiology of ECRS including eosinophilia and lower airway obstruction.

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## Introduction

Chronic rhinosinusitis with nasal polyps (CRSwNP), especially eosinophilic CRS (ECRS), is an intractable inflammatory disease of the upper airway.<sup>1</sup> Although the precise etiology and pathophysiology underlying this disease remain poorly understood, imbalances in local Th1, Th2, Th17, and Treg responses following

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exposure to microbes, including viruses, fungi, and bacteria, appear to be involved.<sup>2,3</sup>

*Staphylococcus aureus* exotoxins, especially staphylococcal enterotoxin B (SEB), are among the best characterized elicitors of cellular responses and are thought to be associated with the pathogenesis of CRSwNP.<sup>4–6</sup>

IL-10 is a major cytokine that regulates both innate and acquired immune responses. IL-10 is considered to be the most important anti-inflammatory cytokine and a key cytokine that prevents inflammation-mediated tissue damage.<sup>7,8</sup> IL-10 acts on antigen-presenting cells to dampen T-cell activation including Th2 cells, and inhibits activation of mast cells and eosinophils.<sup>9</sup> In nasal explant culture, IL-10 promotes expression of clara cell 10-kDa protein, a multifunction protein with anti-inflammatory and immunomodulatory effects.<sup>10</sup> IL-10 inhibits FcεRI-mediated IL-6 and TNF-α production by dendritic cells purified from the nasal mucosa.<sup>11</sup>

Controversial discussions have occurred regarding whether local expression of IL-10 in sinonasal tissues is associated with the pathogenesis of CRSwNP. Levels of IL-10 protein in NPs are similar to levels in inferior turbinates in Belgian patients.<sup>12</sup> On the other hand, varying results were seen in Asian patients including Korean and Chinese individuals.<sup>2,13–16</sup>

Several ex vivo NP models have shown that NPs produce IL-10 in response to various stimuli including *S. aureus*-derived exotoxins and biofilms, cross-linking of IgE, and drugs such as clarithromycin and dexamethasone.<sup>17–21</sup> For example, we have recently reported that *S. aureus*-derived non-superantigenic alpha toxin promotes IL-10 production by dispersed NP cells in a dose- and phase-dependent manner.<sup>18</sup> In addition, another recent report demonstrated that 24-h stimulation with SEB resulted in a significant induction of NP CD4<sup>+</sup> T cells that produce IL-10.<sup>22</sup>

In the present study, we characterized the production of IL-10 by sinonasal tissue cells following exposure to SEB. The present findings provide novel insight into the pathogenesis of ECRS and its regulation by IL-10.

## Methods

### Patients

The study examined 28 Japanese CRSwNP patients (age range 22–71 years; mean age 56.6 years). The presence of CRSwNP was determined based on diagnostic criteria reported in a European position paper on rhinosinusitis and NPs.<sup>23</sup> All patients were resistant to macrolide therapy for at least 3 months, as determined by computed tomography showing persistent abnormal shadows in sinuses. Subsequently, they underwent endoscopic sinus surgery. According to the JESREC (Japanese Epidemiological Survey of Refractory Eosinophilic Chronic Rhinosinusitis) criterion, 20 and 8 patients were diagnosed with eosinophilic CRS (ECRS) and non-eosinophilic CRS (non-ECRS), respectively.<sup>1</sup> For at least 8 weeks prior to surgery, none of the participants received systemic glucocorticoids, and for at least 3 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 (FEV<sub>1</sub>/FVC) ratio were determined. A radiological assessment of the severity of rhinosinusitis in each patient was also performed using the Lund–Mackay system.<sup>23</sup> Sections from surgically excised NPs were stained with hematoxylin/eosin solution, and the average number of eosinophils per high power field (HPF; ×400) was determined. Thirteen patients (age range 28–77 years; mean age 55.4 years) with CRS without NPs (CRSsNP) were

enrolled as controls.<sup>1</sup> 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 and Dentistry.

### Culture of dispersed NP cells and uncinat tissue (UT) cells

NPs and UTs were sampled from patients with CRSwNP and CRSsNP, respectively. Dispersed NP cells and dispersed UT cells were prepared from NPs and UTs, respectively, by enzymatic digestion, as previously described.<sup>18</sup> Briefly, the minced nasal tissues were incubated for 2 h at 37 °C in RPMI 1640 (1 g tissue per 4 mL) containing 2.0 mg/mL protease, 1.5 mg/mL collagenase, 0.75 mg/mL hyaluronidase, and 0.05 mg/mL DNase (Sigma, St. Louis, MO). The cell suspension was then filtered through a 70-μm cell strainer (BD Falcon, Bedford, MA) to remove any undigested tissue and washed two times with washing medium (RPMI 1640 supplemented with 2% FCS (Invitrogen, Carlsbad, CA), 2 mM glutamine, 100 U/ml penicillin, and 100 μg/mL streptomycin (Sigma). The cell pellet was resuspended in erythrocyte lysis buffer (Roche, Indianapolis, IN) and washed with washing medium. After washing, nasal tissue cells were suspended in culture medium (RPMI 1640 supplemented with 10% FCS, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/mL streptomycin). Our previous investigation using cytospin analysis demonstrated that 8.5 ± 5.3%, 11.7 ± 8.9%, 8.9 ± 8.2%, 8.5 ± 6.8%, 7.8 ± 11.1%, 10.9 ± 10.5%, 15.5 ± 6.7%, and 21.6 ± 7.7% cells in NP cells (n = 4) express c-kit, ECP/EPX, CD79α, CD68, CD4, CD8, cytokeratin, and vimentin, respectively.<sup>5</sup> On the other hand, our preliminary data using cytospin analysis showed that 4.3 ± 2.4%, 7.7 ± 5.0%, 17.9 ± 7.4%, 4.1 ± 5.5%, 0.6 ± 1.1%, 2.9 ± 1.8%, 30.9 ± 8.0% and 46.7 ± 3.2% cells in UT cells (n = 3) express CD3, CD19, CD68, c-kit, ECP/EPX, neutrophil elastase, cytokeratin and vimentin, respectively, suggesting that UT cells contain more constitutive cells and less inflammatory cells than NP cells. The cells (1 × 10<sup>6</sup>/mL) were stimulated with 0.01, 0.1, or 1.0 ng/mL SEB (Toxin Technology, Sarasota, FL) and incubated at 37 °C in a 5% CO<sub>2</sub> atmosphere. An aliquot of the culture supernatant was collected after 24 and 72 h and stored at –80 °C for subsequent cytokine analysis. Adherent and non-adherent NP cells were prepared as previously described.<sup>24</sup> Briefly, NP cells were rested in a culture bottle (Sumitomo Bakelite, Tokyo, Japan) at a concentration of 2 × 10<sup>6</sup>/mL in RPMI 1640 culture medium supplemented with 10% heat-inactivated FCS and L-glutamine-penicillin-streptomycin solution. After incubation at 37 °C in a 5% CO<sub>2</sub> atmosphere for 120 min, the non-adherent cells were removed and the adherent cells were collected using a cell scraper (Becton Dickinson Labware, Franklin Lakes, NJ). Our preliminary result showed that 79.7% and 16.4% cells express CD68 in adherent and non-adherent NP cells, respectively. The collected cells were cultured in the presence or absence of 1 ng/mL SEB for 72 h.

### Cytokine measurement

The levels of IL-5, IL-13, IFN-γ, and IL-10 were determined using Opt EIA sets (BD Biosciences: Franklin Lakes, NJ), according to the manufacturer's instructions. The level of IL-17A was determined using a DuoSet ELISA Development Kit (R&D Systems, Minneapolis, MN). The detection limits were 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.

### Immunohistochemistry

Immunohistochemical staining for IL-10 was performed according to a previously described protocol.<sup>6</sup> Briefly, 4-μm sections were collected from paraffin-embedded tissue blocks,

deparaffinized and rehydrated. The sections were heated in sodium citrate buffer (pH 6.5) in a microwave oven for antigen retrieval and were incubated with primary antibodies including goat anti-human IL-10 polyclonal antibody (R&D Systems) or control antibody (Universal Negative Control, Dako Japan, Tokyo, Japan) at 4 °C overnight. Rabbit anti-goat immunoglobulin conjugated with a peroxidase-labelled amino acid polymer (Histofine Simple Stain MAX-PO (G), Nichirei, Tokyo, Japan) was used according to the manufacturer's instructions to detect antibody–antigen interactions. The nuclei of the sections were then stained with hematoxylin and the sections were examined under a light microscope. For double immunohistochemistry, primary monoclonal antibodies of mouse IgG1 were used including anti-CD3 (F7.2.38, Dako), anti-CD19 (LE-CD19, Dako), anti-CD68 (KP1, Dako), anti-tryptase (AA1, MBL Ltd, Nagoya, Japan), anti-ECP/EPX (EG2, Pharmacia), neutrophil elastase (NP57, Dako) and negative control (X0931, Dako). ImmPRESS-AP Reagent (anti-Mouse Ig) and ImmPACT Vector RED (Vector Laboratories, Burlingame, CA) were used to detect antibody–antigen interactions.

### 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. The Kruskal–Wallis test followed by Dunn's test was used for multiple comparisons. Correlation analyses were performed using the Spearman rank correlation. Statistical analyses were performed using GraphPad Prism (GraphPad Software, La Jolla, CA) version 9.2, with  $P < 0.05$  considered to be significant.

## Results

### Production of IL-10 by NP and UT cells in response to SEB

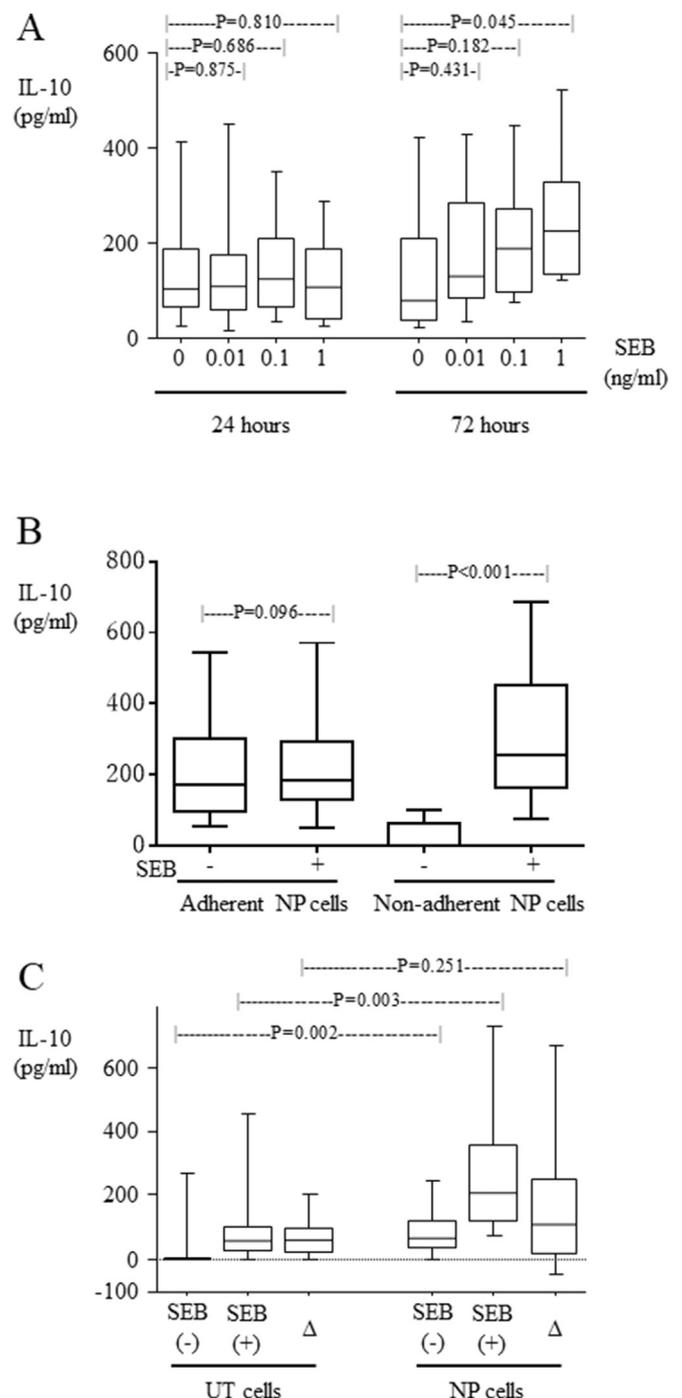
Exposure to SEB for 24 h did not induce a significant increase in IL-10 production by NP cells ( $n = 15$ ,  $P = 0.972$ , Kruskal–Wallis test). On the other hand, dose-dependent significant production was seen following 72-h exposure to SEB ( $P = 0.048$ ). The Dunn's test further revealed that SEB-induced IL-10 production by NP cells was significant at the concentration of 1 ng/mL ( $P = 0.045$ ) (Fig. 1A). Our preliminary result ( $n = 5$ ) showed that all the levels of IL-10 in the culture supernatants were below the detection limit (4 pg/mL) with 0-h stimulation with or without SEB (data not shown). When we separated NP cells into adherent and non-adherent cells, non-adherent NP cells ( $P < 0.001$ ) but not adherent NP cells ( $P = 0.096$ ) produced substantial levels of IL-10 ( $n = 12$ , Fig. 1B).

Baseline production of IL-10 after 72 h in the absence of SEB was significantly higher in NP cells ( $n = 28$ ) than UT cells ( $n = 13$ ) ( $P = 0.002$ ), and the amount of IL-10 in response to SEB was also higher in NP cells than UT cells ( $P = 0.003$ ). However, the degree of IL-10 response to SEB was not significantly different between the cell types ( $P = 0.251$ , Fig. 1C).

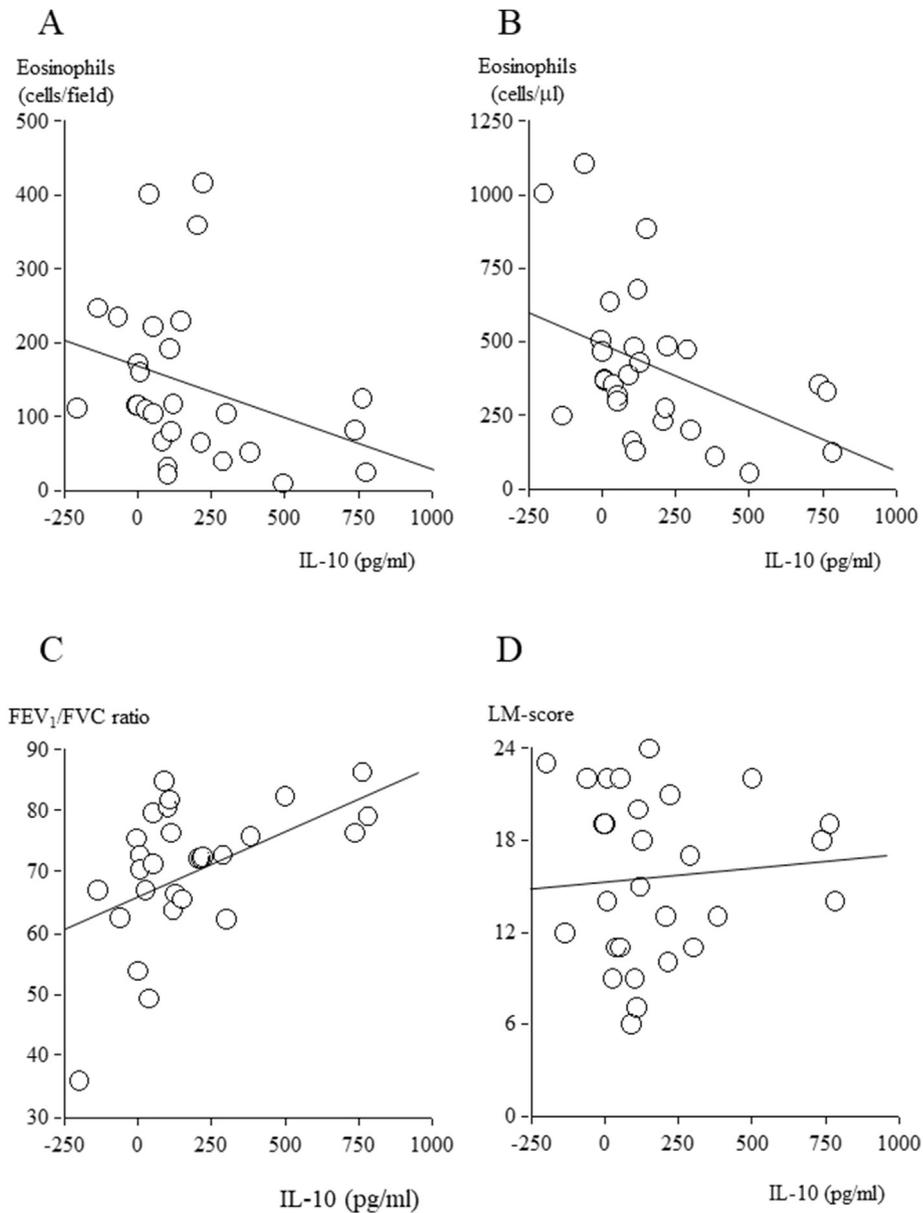
### Pathophysiological significance of SEB-induced IL-10 production by NP cells

We next characterized the degree of IL-10 response to SEB by NP cells regarding the pathophysiology of CRSwNP. The IL-10 responses were significantly and negatively correlated with the degree of eosinophilia in NPs ( $r = -0.401$ ,  $P = 0.029$ , Fig. 2A) and peripheral blood cells ( $r = -0.464$ ,  $P = 0.016$ , Fig. 2B). We grouped NP into eosinophilic ( $\geq 70$  eosinophils/HPF,  $n = 20$ ) or non-eosinophilic ( $< 70$  eosinophils/HPF,  $n = 8$ ) based on the JESREC criterion.<sup>1</sup> The Kruskal–Wallis test showed a significant difference

in the degree of IL-10 response among the groups of UT, non-eosinophilic NP and eosinophilic NP ( $P = 0.023$ ). The Dunn's test further revealed that the level of IL-10 was significantly lower in UT ( $P = 0.036$ ) and eosinophilic NP ( $P = 0.038$ ) compared with non-eosinophilic NP whereas the level was similar between UT and



**Fig. 1.** SEB-induced IL-10 production by sinonasal tissues. 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. (A): IL-10 production by NP cells following exposure to a serial concentration of SEB for 24 and 72 h. P values were determined by Dunn's test. (B): Comparison of SEB-induced IL-10 production between adherent and non-adherent NP cells. P values were determined by Wilcoxon's signed-ranks test. (C): Comparison of SEB-induced IL-10 production between NP cells and UT cells. P values were determined by Mann–Whitney's U test. Δ indicates SEB-specific production which was calculated as amount of IL-10 in response to SEB minus baseline amount.



**Fig. 2.** Relationship between SEB-induced IL-10 production by NP cells and pathophysiology of CRSwNP including NP eosinophilia (A), peripheral blood eosinophilia (B), FEV<sub>1</sub>/FVC ratio (C) and CT score (D).

eosinophilic NP ( $P > 0.999$ ) (Fig. 3A). Conversely, the IL-10 levels were significantly and positively correlated with FEV<sub>1</sub>/FVC ratio ( $r = 0.465$ ,  $P = 0.016$ , Fig. 2C). The levels of IL-10 were not correlated with the radiological severity of rhinosinusitis ( $r = -0.065$ ,  $P = 0.725$ , Fig. 2D).

Patients with CRSwNP were divided into non-ECRS ( $n = 8$ ), mild ECRS ( $n = 3$ ), moderate ECRS ( $n = 7$ ), and severe ECRS ( $n = 10$ ) based on the JESREC criterion.<sup>1</sup> The Kruskal–Wallis test showed a significant difference in the degree of IL-10 response to SEB among the four groups ( $P = 0.012$ ). The Dunn's test further revealed that induction of IL-10 was significantly lower in patients with severe ECRS compared with non-ECRS ( $P = 0.022$ ) (Fig. 3B).

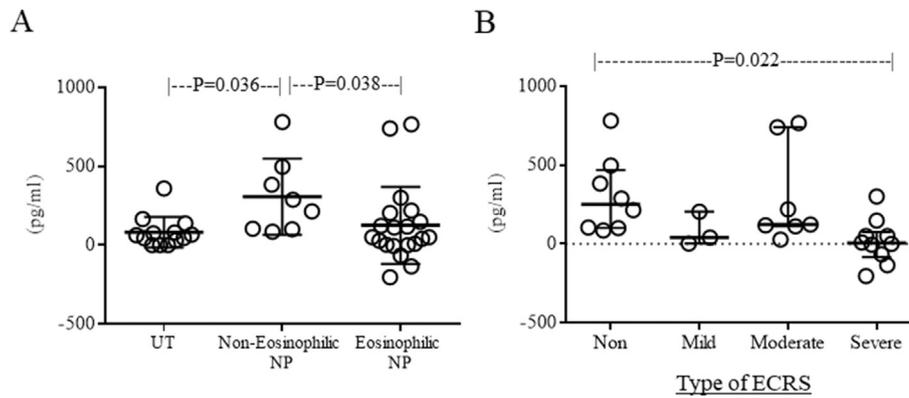
#### Effect of IL-10 blockade on SEB-induced cytokine production by NP cells

SEB induces production of pro-inflammatory cytokines including IL-5, IL-13, IFN- $\gamma$ , and IL-17A by NP cells.<sup>5,6,25,26</sup> To

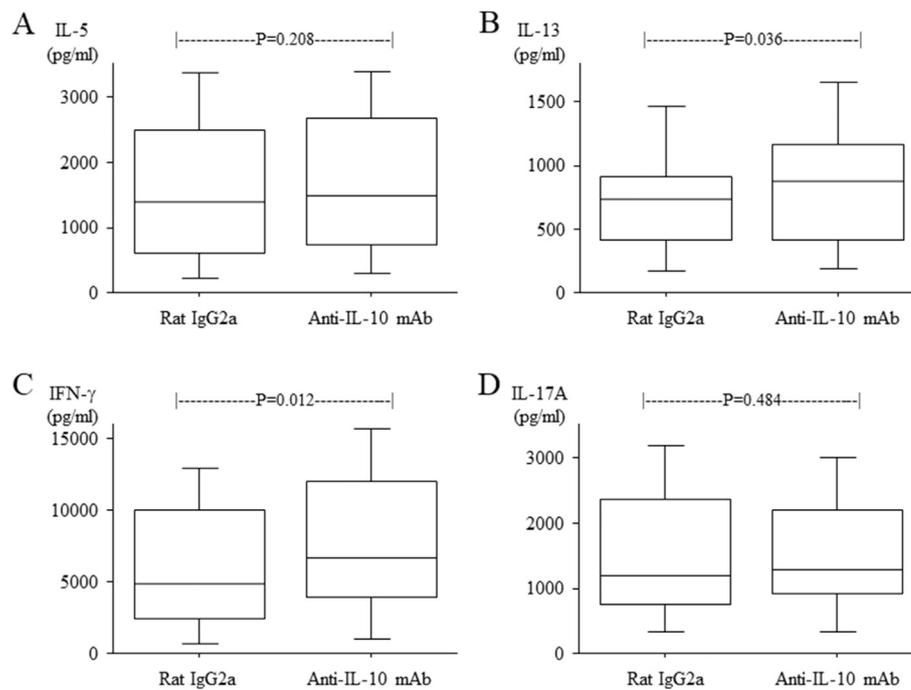
determine the role of IL-10 in the production of pro-inflammatory cytokines by NP cells, the effect of blocking IL-10 activity by treatment with the anti-human IL-10 Ab was assayed. Compared to control rat IgG2a, treatment with anti-human IL-10 Ab significantly augmented SEB-induced production of IL-13 ( $P = 0.036$ ) and IFN- $\gamma$  ( $P = 0.012$ ), but not IL-5 ( $P = 0.208$ ) or IL-17A ( $P = 0.484$ ) (Fig. 4).

#### Local expression of IL-10 in NPs

The expression and distribution of IL-10 in the NPs of CRSwNP patients were immunohistochemically examined. Representative staining showed that IL-10 protein was mainly expressed in infiltrating inflammatory cells (Fig. 5). Number of NP eosinophils was significantly higher in ECRS (median: 142 cells/HPF,  $n = 20$ ) than non-ECRS (median: 36 cells/HPF,  $n = 8$ ), showing a statistical significance between the groups ( $P < 0.001$  by Mann–Whitney U test). Our preliminary results using double immunohistochemistry ( $n = 10$ ) revealed that IL-10 expression was seen in a variety of cells



**Fig. 3.** Characterization of SEB-induced IL-10 production by NP cells based on the JESREC criterion. **(A):** Comparison of SEB-induced IL-10 production between eosinophilic and non-eosinophilic NP cells. P values were determined by Mann–Whitney's U test. **(B):** Comparison of SEB-induced IL-10 production and disease severity of ECRS. P values were determined by Dunn's test.



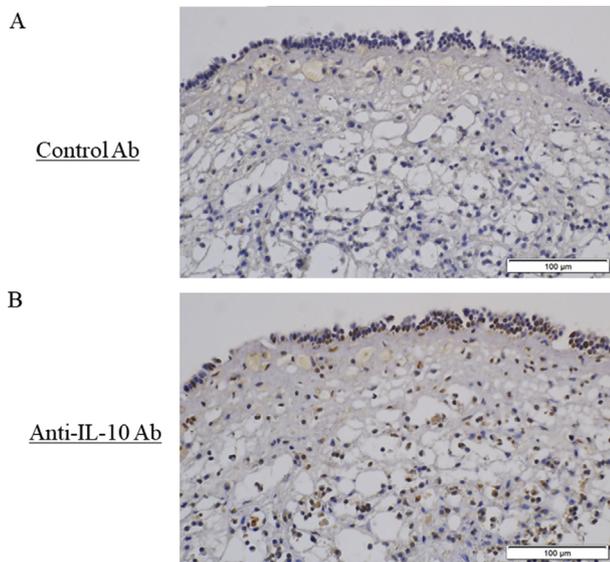
**Fig. 4.** Effect of IL-10 neutralization on SEB-induced cytokine production by NP cells. NP cells were stimulated with SEB in the presence of either anti-human IL-10 mAb or control rat IgG2a for 72 h, then levels of IL-5 **(A)**, IL-13 **(B)**, IFN- $\gamma$  **(C)** and IL-17A **(D)** in culture supernatants were determined. P-values were determined using Wilcoxon signed-rank test.

including CD3<sup>+</sup>, CD19<sup>+</sup>, CD68<sup>+</sup>, tryptase<sup>+</sup>, ECP/EPX<sup>+</sup>, and neutrophil elastase<sup>+</sup> cells in NP, suggesting that T cells, B cells, macrophages, mast cells, eosinophils and neutrophils can express IL-10 in NP. The positive rate of IL-10 expression in each cell was not statistically different between patients with ECRS (n = 5) and non-ECRS (n = 5).

## Discussion

In the present study, we characterized SEB-induced IL-10 production by NP cells. We found that eosinophilic NPs produced significantly less IL-10 than non-eosinophilic NPs. In addition, the amount of IL-10 was significantly and negatively correlated with the degree of local and peripheral blood eosinophilia, and was positively correlated with lower airway obstruction. Our results suggest that impaired IL-10 production following exposure to SEB closely affects the pathophysiology of ECRS.

IL-10 is expressed in infiltrating inflammatory cells in NPs. IL-10 is produced by a variety of cells including macrophages, dendritic cells, monocytes, T cells, and B cells.<sup>8</sup> T cells are the principal source of allergen-induced IL-10 production in NPs.<sup>27</sup> In addition, exposure to SEB results in a significant induction of CD4<sup>+</sup> T cells that produce IL-10 in NP cells.<sup>22</sup> Consistent with these observations, non-adherent, but not adherent, NP cells produced IL-10 in response to SEB, suggesting that non-adherent cells in NPs such as T cells and B cells are the primary source of IL-10 in response to SEB. We previously showed that adherent NP cells produce IL-10 in response to staphylococcal protein A.<sup>24</sup> These results suggest that different cell types in NPs can react with different components of *S. aureus* to produce IL-10. Although impaired IL-10 production by NP cells associated with pathophysiology of ECRS including eosinophilia, further investigation is desired whether impairment of IL-10 production from non-adherent cells by SEB is critical or not to pathophysiology of ECRS.



**Fig. 5.** Immunohistochemical staining of IL-10 in NP. Sections were reacted with goat polyclonal antibody against IL-10 (B), or control (A) after which they were stained using the avidin-biotin-immunoperoxidase system.

Baseline production of IL-10 was significantly higher in NP cells than UT cells. Our results are similar to the finding by Xu *et al.* who showed that the expression of IL-10 protein and mRNA was significantly higher in NPs than UTs.<sup>16</sup> Various results have been found regarding whether the expression of IL-10 is increased in NPs.<sup>2,12–16,28</sup> This may be due to the different sampling methods (e.g., tissues and fluids) and/or control tissues (e.g., inferior turbinates and sinus mucosae). Production of IL-10 may be induced in the inflammatory milieu as a counter-regulator to limit tissue damage by inhibiting the effects of inflammatory cells such as eosinophils in CRSwNP.<sup>29</sup>

Long-term exposure to SEB and antigen upregulates local IL-10 expression in mouse models of CRS.<sup>30</sup> On the other hand, no significant difference was seen in IL-10 response to SEB between UT cells and NP cells. Thus, acquired local responsiveness in IL-10 production following exposure to SEB may be sufficient to prevent NP formation in patients with CRSsNP.

The levels of SEB-induced IL-10 production by NP cells were significantly and negatively correlated with the degree of NP eosinophilia, and SEB-induced IL-10 production was significantly lower in eosinophilic NPs compared with non-eosinophilic NPs. This observation is consistent with our previous finding of a negative correlation between NP eosinophilia and production of IL-10 in response to alpha-toxin.<sup>18</sup> In general, IL-10 has an inhibitory effect on eosinophil survival.<sup>29</sup>

IL-10 plays a critical role in controlling eosinophilic airway inflammation.<sup>9</sup> In CRS patients, IL-10 suppresses IL-6 and TNF- $\alpha$  production by upper airway dendritic cells.<sup>11</sup> Additionally, IL-10 suppresses allergen-induced IL-5 and IFN- $\gamma$  production by NP cells.<sup>27</sup> These results suggest that the induction of IL-10 following exposure to both superantigenic and non-superantigenic exotoxins from *S. aureus* plays an important role in preventing eosinophilic inflammation in patients with CRSwNP. Additionally, unlike with alpha-toxin, the levels of IL-10 production induced by SEB in NP cells were significantly and negatively correlated with peripheral blood eosinophilia, suggesting that impaired IL-10 production occurs following exposure to SEB, rather than alpha-toxin, which affects systemic activation of eosinophils.

The definition of ECRS and/or eosinophilic NPs varies among countries and investigators.<sup>2,31–35</sup> For example, the cut-off for the number of tissue eosinophils per HPF ( $\times 400$  magnification) ranges from 5 to 350.<sup>32–35</sup> One study showed no significant difference in IL-10 mRNA expression in NPs between ECRS and non-ECRS patients when ECRS was defined as an eosinophil count of more than 50 per HPF.<sup>36</sup> We have recently proposed a JESREC criterion that defines refractory ECRS.<sup>1</sup> In this criterion, a cut-off value of 70 eosinophils per HPF was associated with the most significant difference in the risk of recurrence. CRSwNP was divided into non-ECRS, mild ECRS, moderate ECRS, and severe ECRS based on the factors of an ethmoid-dominant shadow in computed tomography,  $>5\%$  eosinophils in peripheral blood, and the comorbidities of bronchial asthma and/or aspirin intolerance. The present study showed that SEB-induced IL-10 production was significantly lower in patients with severe ECRS compared with non-ECRS, suggesting that impaired IL-10 production is associated with the severity of CRSwNP. A positive correlation between the levels of IL-10 and FEV<sub>1</sub>/FVC ratio may support this because the presence of asthma and/or aspirin intolerance strongly affects the severity of the disease according to the JESREC criterion.<sup>1</sup> Role of IL-10 in the pathophysiology of asthma has been investigated.<sup>37,38</sup> For example, IL-10-producing CD4<sup>+</sup>CD45RO<sup>+</sup> cells in the peripheral blood are decreased in severe unstable asthmatics compared to not only the mild group but also the severe stable asthmatics.<sup>37</sup> Serum level of IL-10 was diminished in asthmatics than in controls, and a strong negative correlation was found between serum IL-10 and IL-33 levels.<sup>38</sup>

IL-13 is a type 2 cytokine that activates immune cells such as macrophages, B cells, eosinophils, and mast cells as well as non-immune cells such as epithelial cells, endothelial cells, and fibroblasts to induce recruitment of eosinophils and Th2 cells, IgE-mediated reactions, mucous secretions, and remodeling via perostin production.<sup>39,40</sup> We observed that IL-10 blockade led to an increase in SEB-induced IL-13 and IFN- $\gamma$  production. This result is consistent with the previous finding that IL-10 inhibits type 1 cytokine production by reducing synthesis of IL-12 by antigen-presenting cells.<sup>41</sup> IFN- $\gamma$  suppresses type 2 immune responses, although the role of IFN- $\gamma$  in eosinophilic airway inflammation remains controversial.<sup>42</sup> For example, eosinophil-derived IFN- $\gamma$  induces airway inflammation in the absence of lymphocytes.<sup>43</sup> Another study demonstrated that IFN- $\gamma$  disrupts epithelial barrier function in CRS.<sup>44</sup> Therefore, the association between disease severity and impaired IL-10 production following exposure to SEB may be due to the failure of IL-10 to decrease IL-13 and IFN- $\gamma$  production.

In conclusion, although SEB is both harmful and beneficial (protective) to the pathogenesis of ECRS, impairment of IL-10 production, in other word imbalanced production between proinflammatory and anti-inflammatory cytokines, following exposure to SEB may exacerbate the pathophysiology of ECRS and lower airway obstruction. Our observations may provide a rationale for the development of novel therapeutic and preventative approaches that target IL-10 to manage eosinophilic airway diseases such as CRSwNP, allergic rhinitis, and bronchial asthma.

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#### Conflict of interest

The authors have no conflict of interest to declare.

### Authors' contributions

TakeH, MO, and KN designed the study and wrote the manuscript. TF, SK, TakaH contributed to patient collection and ex vivo study. SM contributed to experiments regarding IL-10 neutralization. KK and RF contributed to immunohistochemistry. YN and SI performed the statistical analysis and interpretation of the results.

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