

Letter to the Editor

Regulation and characterization of IL-17A expression in patients with chronic rhinosinusitis and its relationship with eosinophilic inflammation

To the Editor:

IL-17A is a proinflammatory cytokine synthesized by T_H17 cells.¹ Although several reports have demonstrated that IL-17A expression levels in sputum and lung tissue correlated with the severity of asthma symptoms, it remains unclear as to whether IL-17A is involved in the pathogenesis of severe chronic upper airway diseases, including chronic rhinosinusitis with nasal polyps (CRSwNPs).^{2,3} We investigated the local production and regulation of IL-17A in nasal polyps (NPs) by using a recently developed *ex vivo* system.⁴ In addition, local expression of IL-17A was compared among various chronic rhinosinusitis (CRS) phenotypes, and the role of IL-17A in the pathogenesis of CRS was discussed. Details on the methods are available in the Methods section of this article's Online Repository at www.jacionline.org.

Staphylococcus aureus enterotoxin B (SEB) is involved in the pathogenesis of CRSwNPs.⁵ SEB stimulation of dispersed nasal polyp cells (DNPCs) induced significant IL-17A synthesis

(Fig 1, A), suggesting that SEB exerts its pathogenic effects in patients with CRSwNPs through induction of IL-17A synthesis. Neutralization with IL-10 or IL-18 did not affect the SEB-induced IL-17A synthesis. In contrast, neutralization with IL-12/IL-23 p40 partially, albeit significantly, suppressed this synthesis. IFN- γ neutralization significantly improved IL-17A synthesis (Fig 1, B). These results suggest that production of IL-17A is positively and negatively regulated by p40 and IFN- γ , respectively.

The COX pathway also plays important roles in the pathogenesis of CRSwNPs.⁴ Treatment with diclofenac caused a significant decrease in IL-17A production (Fig 1, A). Conversely, SEB-induced IL-17A production in diclofenac-treated DNPCs was enhanced by 21.6%, 45.8%, and 38.8% in the presence of prostaglandin (PG) E_2 administered at 10^{-8} , 10^{-7} , and 10^{-6} mol/L, respectively, when compared with control buffer treatment ($P = .001$). PGE₂, 10^{-7} and 10^{-6} mol/L, significantly increased IL-17A production compared with 10^{-8} mol/L PGE₂, indicating that the effect of PGE₂ was dose dependent, and the highest enhancement was achieved at 10^{-7} mol/L (Fig 1, C). This result suggests that COX metabolism, especially PGE₂, displays a proinflammatory role during SEB-induced IL-17A production.

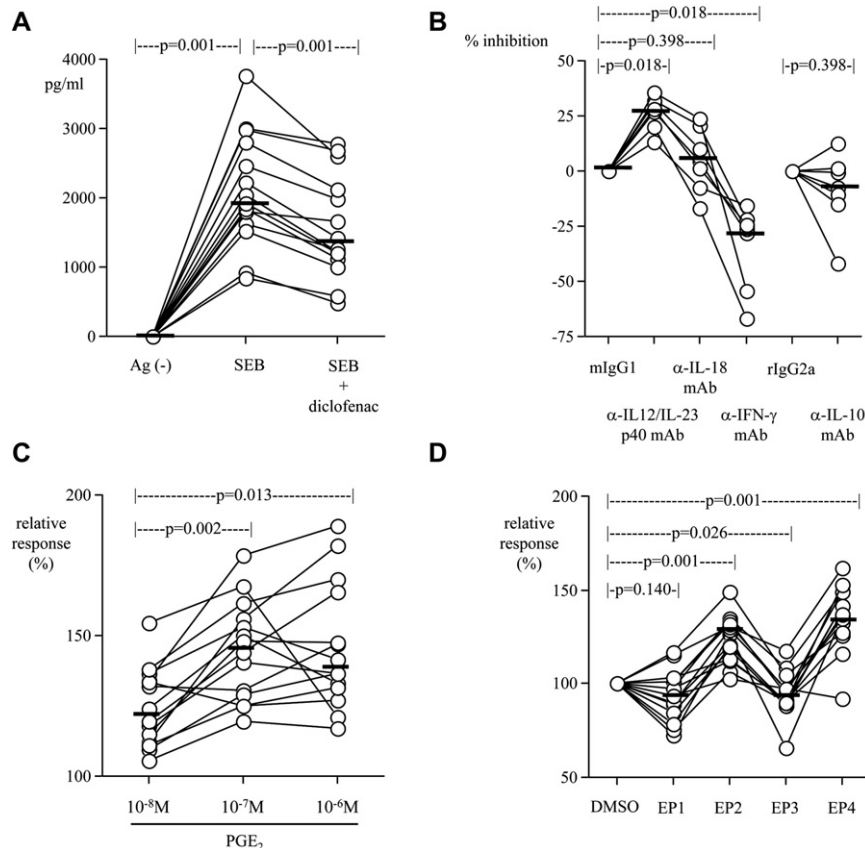


FIG 1. Production and regulation of IL-17A in response to SEB by DNPCs. DNPCs were stimulated with or without SEB (A). Effects of diclofenac (Fig 1, A); neutralization of IL-12/IL-23 p40, IL-18, IFN- γ , and IL-10 (B); PGE₂ (C), and EP receptor-selective agonists (D) on SEB-induced IL-17A production by DNPCs are shown. The bar indicates the median. *P* values were determined by using the Wilcoxon signed-rank test. DMSO, Dimethyl sulfoxide.

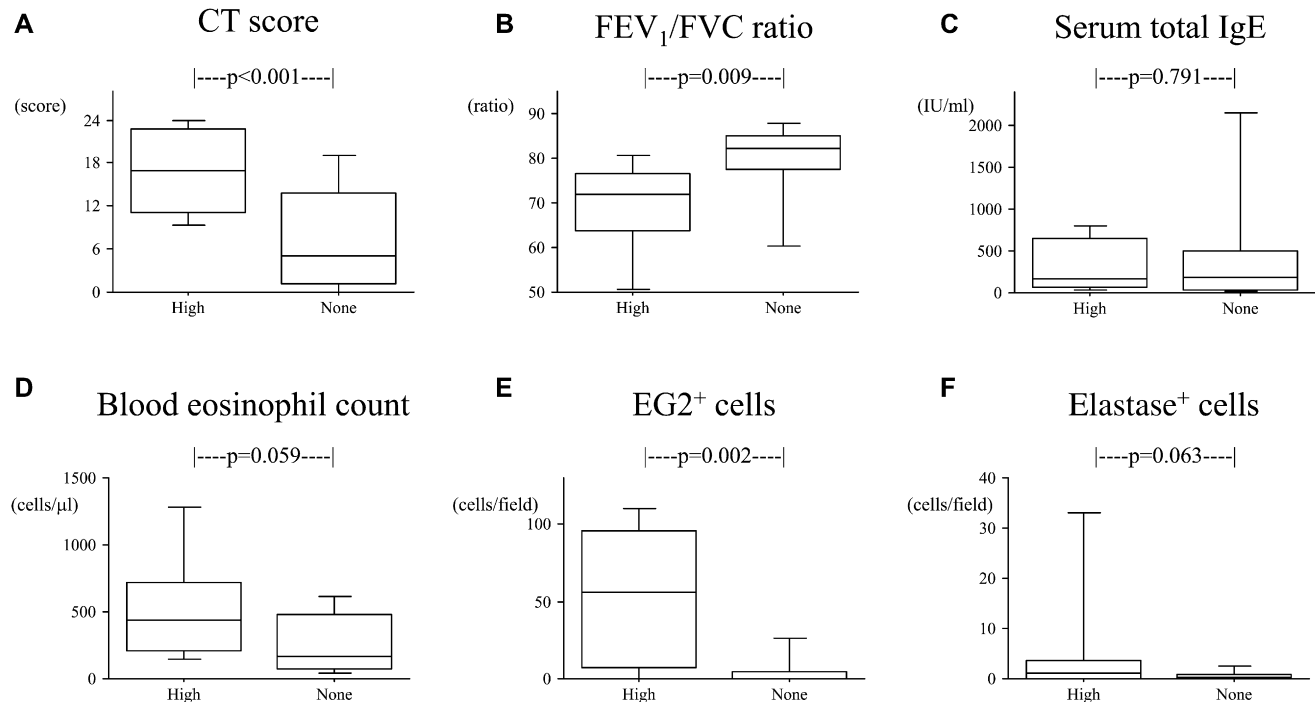


FIG 2. Comparison of pathophysiological characterizations, including radiological severity of sinusitis (A), FEV₁/FVC ratio (B), serum total IgE level (C), blood eosinophil count (D), number of infiltrating eosinophil cationic protein/eosinophil protein X (ECP/EPX)⁺ cells in sinonasal tissues (E), and number of elastase⁺ cells in sinonasal tissues (F), between patients with high numbers of IL-17A⁺ cells in sinonasal tissues and those with none. Rectangles include the range from 25th to 75th percentiles, horizontal lines indicate the median, and vertical lines indicate the 10th to 90th percentiles. P values were determined by using the Mann-Whitney U test.

The inhibition by p40 neutralization was also seen in the presence of diclofenac ($P = .028$), suggesting the additive inhibitory activity by COX inhibition and p40 neutralization.

Treatment with EP1 receptor-selective agonists did not affect the SEB-induced IL-17A synthesis. However, treatment with EP2 and EP4 receptor-selective agonists did significantly augment production in a dose-dependent fashion, and maximum enhancement was achieved at 10^{-6} mol/L (Fig 1, D). In contrast, treatment with EP3 receptor-selective agonist modestly inhibited production. To the best of our knowledge, the present study represents the first report showing that PGE₂ enhances IL-17A production through EP2/EP4 receptor signaling in the human airway.⁶

We immunohistochemically examined the expression and distribution of IL-17A in NPs and uncinate process mucosae (UPM). IL-17A was mainly expressed in infiltrating inflammatory cells. Expression was observed in both NPs and UPM from not only patients with CRS but also patients without CRS, with a greater expression observed in the NPs compared with the UPM (see Fig E1 in this article's Online Repository at www.jacionline.org). Both the number ($P < .001$) and percentage ($P = .002$) of IL-17A⁺ cells in the NPs were significantly higher than those in the UPM (see Fig E2, A and B, in this article's Online Repository at www.jacionline.org). The expression levels were similar in the UPM, regardless of the presence or absence of CRS. In the NPs the percentage ($P = .002$) of IL-17A⁺ cells was significantly higher in asthmatic patients compared with that seen in nonasthmatic patients (see Fig E2, C and D). The percentage was significantly higher in patients with aspirin-induced asthma compared with that seen in patients with aspirin-tolerant asthma ($P = .017$;

see Fig E2, D). CD68⁺ cells (see Fig E3, A-D, in this article's Online Repository at www.jacionline.org), CD4⁺ cells (see Fig E3, E-H), and EG2⁺ cells (see Fig E3, I-L) expressed IL-17A. In contrast, tryptase-positive cells (see Fig E3, M-P), CD8⁺ cells, CD79a⁺ cells, or elastase-positive cells (data not shown) did not express IL-17A.

A moderate but significant positive correlation between the number of IL-17A⁺ cells in the sinonasal tissues and radiologic severity of sinusitis was observed, suggesting that the local expression of IL-17A is positively associated with mucosal inflammation in patients with CRS ($r = 0.389$; $P < .001$; see Fig E4, A, in this article's Online Repository at www.jacionline.org). In contrast, a moderate but significant inverse correlation between the number and FEV₁/forced vital capacity (FVC) ratio was observed ($r = -0.324$; $P = .005$; see Fig E4, B). The number of IL-17A⁺ cells was not correlated with total serum IgE levels ($r = 0.007$, $P = .981$) or blood eosinophil counts ($r = 0.187$; $P = .113$; see Fig E4, C and D). The numbers were not different between the atopic and nonatopic groups ($P = .861$). On the other hand, the degree of EG2⁺ cell infiltration into the sinonasal tissues was moderately but significantly and positively correlated with the number of IL-17A⁺ cells ($r = 0.377$; $P = .002$; see Fig E4, E). This result is consistent with the recently published report by Saito et al⁷ and suggests that the expression of IL-17A is associated with eosinophilic inflammation in patients with CRS. No correlation was observed between the degree of elastase-positive cell infiltration and the number of IL-17A⁺ cells ($r = 0.174$; $P = .271$; see Fig E4, F). Similar results were obtained that patients with high numbers of IL-17A⁺ cells in sinonasal tissues (>75th

percentile [>4.95 cells/field], $n = 19$) showed a significantly severe computed tomographic score ($P < .001$), significantly lower FEV₁/FVC ratio ($P = .009$), and significantly higher EG2⁺ cell counts ($P = .002$) compared with those with no IL-17A⁺ cells ($n = 15$, Fig 2).

It remains unclear as to how IL-17A drives eosinophilic inflammation. Thus we aimed to determine the direct effects of recombinant IL-17A on cytokine production by DNPCs. Recombinant IL-17A induced small but significant IL-6 ($P = .012$) and GM-CSF ($P = .012$) production in DNPCs (see Fig E5 in this article's Online Repository at www.jacionline.org). Recombinant IL-17A did not induce IL-5 ($P = .916$), IL-13 ($P = .715$), RANTES ($P = .263$), or eotaxin ($P > .999$) synthesis. Our results might suggest that one of the mechanisms underlying the positive correlation between the local expression of IL-17A and eosinophilia in patients with CRSwNPs is induction of GM-CSF and IL-6 production by IL-17A.^{8,9} Detailed discussions are available in the Discussion section in this article's Online Repository at www.jacionline.org.

The present study provides evidence that IL-17A expression is associated with eosinophilic inflammation of CRSwNPs both *in vivo* and *ex vivo*. Although comparison of different tissues (eg, NPs vs nasal mucosa), measurement of IL-17A levels in nasal lavage fluid, and characterization of other members of the IL-17 family should be made in future investigations, these observations might provide a basis for future therapeutic approaches targeting not only IL-17A but also regulatory cytokines, PGE₂, and EP receptor-selective agonists in the management of severe eosinophilic airway diseases.

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METHODS

Patients and tissue sampling

Sixty-four Japanese patients with CRS were enrolled in the study. Briefly, 48 of the 64 patients with CRS exhibited NPs (the CRSw/NPs group). The remainder of the patients with CRS demonstrated no visible polyps in the middle meatus (the CRSw/oNPs group, $n = 16$). The patients entered into the CRSw/NPs and CRSw/oNPs groups were defined by using the diagnostic criteria reported by Benninger et al.^{E1} Thirty-two patients were asthmatic and had NPs. Of these, 17 were thought to exhibit aspirin sensitivity based on their history of asthma attacks precipitated by nonsteroidal anti-inflammatory drugs (aspirin-intolerant asthma group). The remainder of the asthmatic patients were grouped into the aspirin-tolerant asthma group ($n = 15$). All patients were resistant to medical treatment, including macrolide therapy, and thus underwent endonasal sinus surgery. During surgery, the NPs and UPM were sampled from the patients in the CRSw/NPs and CRSw/oNPs groups, respectively, and as described previously.^{E2} None of the participants received systemic steroid treatment for a period of at least 8 weeks before surgery, and none received pharmacotherapy for sinusitis, such as macrolide antibiotics, antihistamines, antileukotrienes, nonsteroidal anti-inflammatory drugs, or intranasal steroids for a period of at least 3 weeks before surgery. None of the participants had immunodeficiency, genetic illness, or other causes of nonidiopathic CRS. Before surgery, we examined total serum IgE levels, blood eosinophil counts, and FEV₁/FVC ratios in each patient. Computed tomographic examination was performed to evaluate the radiologic severity of sinusitis, and the severity was graded according to the Lund-Mackay system for each patient.^{E3} The laboratory testing was usually performed 1 month before surgery. In addition, 12 patients without CRS with normal UPM at inspection and blowout fracture, optic canal fracture, and sphenoidal/posterior ethmoidal cyst were enrolled in the study. The clinical characteristics of the patients are presented in Table E1 of this article's Online Repository at www.jacionline.org. All patients provided informed consent before their participation, and the study was preapproved by the Human Research Committee of the Okayama University Graduate School of Medicine and Dentistry.

Immunohistochemistry

We performed immunohistochemical staining for IL-17A, eosinophils, and neutrophils (Table E2 of this article's Online Repository at www.jacionline.org), according to a previously described protocol.^{E4} Briefly, 4- μ m sections were collected from paraffin-embedded tissue blocks, deparaffinized, and rehydrated. The sections were incubated with trypsin for antigen retrieval and incubated with primary antibody, including 1:50 diluted rabbit anti-human IL-17A polyclonal antibody (H-132; Santa Cruz Biotechnology, Santa Cruz, Calif) or control serum (Universal Negative Control; Dako Japan, Tokyo, Japan) at 4°C overnight. A Histofine MAX-PO (Nichirei Bioscience, Tokyo, Japan) with diaminobenzidine substrate was used according to the manufacturer's instructions to detect antibody-antigen interactions. The sections were then nuclear stained with hematoxylin and examined with a light microscope. Positively stained cells, except for mucosal constitutive cells, including epithelial cells, goblet cells, glands, fibroblasts, and vascular endothelial cells, were determined as infiltrating inflammatory cells and were counted in 5 fields at high power in which the highest cellular infiltration was observed. The mean number of positive cells was then determined.

Double immunofluorescence

To determine IL-17A expression, double immunohistochemistry was performed, as described previously.^{E4} Briefly, sections were incubated with antibodies directed against markers of mast cells, eosinophils, neutrophils, B cells, macrophages, T cells, epithelial cells, vascular endothelial cells, and fibroblasts or control antibody (Universal Negative Control [mouse], Dako), together with either rabbit anti-human IL-17A antibody or control antibody (Universal Negative Control [rabbit], Dako) overnight at 4°C (Table E2 of this article's Online Repository at www.jacionline.org). After washing, sections were further incubated with Alexa-Fluor 488-conjugated goat anti-mouse IgG (Invitrogen, Eugene, Ore) diluted 1:200 and Alexa-Fluor 546-conjugated goat anti-rabbit IgG (Invitrogen) diluted 1:200 for 2 hours at 37°C. The sections were then observed with a confocal laser scanning microscope (Zeiss, Hallberg, Germany).

Culture of DNPCs

DNPCs were prepared from 14 NPs by means of enzymatic digestion, as described previously.^{E4} DNPCs (500 μ L, 2×10^6 /mL) were cultured in flat-bottom, 48-well culture plates (AGC Techno Glass, Tokyo, Japan) in the presence or absence of 1 ng/mL SEB (Toxin Technology, Sarasota, Fla) at 37°C in a 5% CO₂/air mixture. The culture supernatant was collected after 72 hours, and the IL-17A concentration was calculated. To determine the cytokines that regulated SEB-induced IL-17A production, anti-human mAbs for IL-12/IL-23 p40 (C8.6, murine IgG1; BD Biosciences, San Jose, Calif), anti-human IL-18 (125-2H, murine IgG1; MBL, Nagoya, Japan), anti-human IFN- γ (B27, murine IgG1; BD Biosciences), and anti-human IL-10 (JES3-19F1, rat IgG2a; BD Biosciences) at 10 μ g/mL or the respective control IgG (murine IgG1 from R&D Systems, Minneapolis, Minn, and rat IgG2a from BD Biosciences) were added to the cultures. The concentration of antibodies in our neutralization experiments was determined according to the manufacturers' instructions. DNPCs were stimulated with SEB in the presence or absence of 10^{-5} mol/L diclofenac sodium (Wako Pure Chemicals, Osaka, Japan) to determine the role of COXs. Diclofenac-treated DNPCs were cultured with SEB along with serial dilutions of PGE₂ (Cayman, Ann Arbor, Mich) or control buffer (dimethyl sulfoxide, Wako) to determine the effects of PGE₂ on IL-17A production. Alternately, EP-selective agonists or control buffer (0.05% dimethyl sulfoxide) were added to the cultures. The receptor-selective agonists for EP1 (ONO-DI-004), EP2 (ONO-AE1-259-01), EP3 (ONO-AE-248), and EP4 (ONO-AE1-329) were provided by Ono Pharmaceuticals (Osaka, Japan).

Effects of IL-17A on eosinophilia-associated cytokine production by DNPCs

Cells were stimulated with 5 or 20 ng/mL human recombinant IL-17A or control buffer (800 nmol/L HCl) for 72 hours to determine whether IL-17A directly induces eosinophilia-associated cytokine production in DNPCs. The concentrations of IL-5, IL-6, IL-13, GM-CSF, RANTES, and eotaxin in the supernatant were then determined. Relative production was estimated by dividing each amount induced by IL-17A into the amount for control buffer.

Measurement of cytokines

The levels of IL-5, IL-6, IL-13, and eotaxin in the culture supernatants were measured with Opt EIA sets (BD Biosciences), according to the manufacturer's instructions. The levels of RANTES, GM-CSF, and IL-17A were measured with a DuoSet ELISA development kit (R&D Systems). The detection limit of these assays was 4 pg/mL for IL-5, 2 pg/mL for IL-6, 2 pg/mL for IL-13, 2 pg/mL for GM-CSF, 4 pg/mL for RANTES, 8 pg/mL for IL-17A, and 4 pg/mL for eotaxin.

Statistical analysis

Values are presented as medians. A nonparametric Mann-Whitney U test was used for comparing data between groups, and the Wilcoxon signed-rank test was used for analysis within groups. A correlation analysis was performed by using the Spearman correlation coefficient by rank. P values of less than .05 were considered statistically significant. Statistical analyses were performed with StatView software (version 4.5; Abacus Concepts, Berkeley, Calif).

DISCUSSION

In the present study we examined the regulation and characterization of IL-17A expression during CRS pathogenesis. Results suggest that the expression of IL-17A in sinonasal tissue is associated with not only local eosinophilic inflammation and radiologic severity of sinusitis but also lower airway obstruction. *Ex vivo* analysis revealed that DNPCs produce IL-17A in response to SEB and that the production is regulated by IL-12/IL-23 p40 and IFN- γ . In addition, results also suggest that PGE₂ exerts its

proinflammatory effects on IL-17A production through coupling to EP2 and EP4 receptors.

DNPCs synthesized IL-17A in response to SEB. IL-17A secretion by skin-infiltrating T cells from patients with atopic eczema is known to be triggered by SEB.^{E5} However, little is known regarding whether SEB is able to induce IL-17A synthesis in airway allergic/eosinophilic diseases, despite exposure to SEB-induced eosinophilic inflammation.^{E6,E7} These results were not consistent with recently published results of Xu et al.^{E8} These authors demonstrated that SEB did not induce IL-17A production in NP explants. This discrepancy might have arisen from the differences in the materials used in the present study, such as the tissue treatment and the populations tested. Recent evidence also suggests that SEB plays an important role in the pathogenesis of CRSwNPs.^{E9-12} *Staphylococcal enterotoxin (SE)* and SE-producing *Staphylococcus aureus* have been detected at high levels in NP specimens.^{E10,E11} Lymphocytes in NPs have also been shown to demonstrate skewing in the V beta domains and a strong association with SE.^{E12} Thus our present results suggest that SEB exerts its pathogenic effects through the induction of IL-17A synthesis, especially in patients with CRSwNPs.

SEB-induced IL-17A production in DNPCs was both positively and negatively regulated by IL-12/IL-23 p40 and IFN- γ , respectively. We were unable to determine whether the effects of p40 neutralization were mediated through IL-12 or IL-23. Because neutralization of IL-12/IL-23 p40 and IFN- γ showed opposite effects on IL-17A production, respectively, the effects of p40 neutralization are thought to be mainly mediated through IL-23, and the production of IFN- γ usually occurs downstream of IL-12 and through the activation of signal transducer and activator of transcription 4.^{E13} IL-23 plays a crucial role in the differentiation, acquisition, and maintenance of T_H17 cells and enhances T_H2 cell-mediated eosinophilic airway inflammation.^{E14,E15} In contrast, several studies have reported that IFN- γ repressed the production of IL-17A and the differentiation of T_H17 cells.^{E16,E17} The present findings are consistent with these reports and suggest that production of IL-17A in the human airway might also be regulated by IL-23 and IFN- γ .

Interestingly, treatment with diclofenac led to inhibition of SEB-induced IL-17A production by DNPCs, an effect that was reversed after the addition of PGE₂. Diclofenac inhibited IL-17A production in a dose-dependent fashion, and significant inhibitions were seen at the concentration of 10⁻⁷ to 10⁻⁴ mol/L (data not shown). This result suggests that COX metabolism, especially PGE₂, displays a proinflammatory role during SEB-induced IL-17A production. Several reports have recently shown that PGE₂ promotes IL-17A production and T_H17 differentiation.^{E18-E20} TCR triggering in the presence of PGE₂ increases IL-17A production in human memory CD4⁺ T cells.^{E18} In a murine model of arthritis, PGE₂ played an important role in IL-23-induced neutrophil migration by enhancing IL-17A synthesis.^{E20} To the best of our knowledge, the present study represents the first report showing that PGE₂ enhances IL-17A production in the human airway.

Experiments with EP receptor-selective agonists suggest that enhancement of SEB-induced IL-17A production by PGE₂ is mediated by EP2/EP4 receptor signaling. Signaling through EP2/EP4 receptors is able to induce proinflammatory effects, including the promotion and maturation of Langerhans cells, and mucinous component (MUC) AC production in airway epithelial cells.^{E21,E22} SEB-induced IL-17A synthesis by DNPCs was

conversely inhibited by EP3 receptor-selective agonists. In a murine model of asthma, EP3-deficient mice displayed severe eosinophilic inflammation and production of T_H2 cytokines, such as IL-5 and IL-13.^{E23} These results suggest that signaling through EP2/EP4 and EP3 receptors exhibit the opposite effects on IL-17A production.

The results in the present study are not consistent with those of our previously published report.^{E4} In our previous report PGE₂ exhibited an anti-inflammatory role during the SEB-induced production of the eosinophilia-associated cytokines IL-5, IL-13, and RANTES. EP2 receptor-selective agonists strongly inhibited the production of all of these cytokines. EP3 and EP4 receptor-selective agonists partially suppressed these responses, whereas EP1 receptor-selective agonists did not. These results might suggest that PGE₂ exerts opposite roles during the regulation of T_H2 and T_H17 cytokine production in the airways.

The number of cells expressing IL-17A in sinonasal tissues is significantly higher in the CRSwNPs group compared with those in the non-CRS and CRS without NPs (CRSwONPs) groups. Age and sex were not significantly different among the groups. Blood eosinophil counts were significantly higher in the CRSwNPs group compared with those in the non-CRS group ($P < .001$) and the CRSwONPs group ($P = .016$). Levels of serum total IgE were significantly higher in the CRSwNPs group compared with those in the non-CRS group ($P = .029$). Conversely, the FEV₁/FVC ratio was also significantly lower in the CRSwNPs group compared with that in the non-CRS group ($P = .003$). However, the presence of a high serum total IgE level (>170 IU/mL), high blood eosinophilia (>500 counts/ μ L), or low FEV₁/FVC ratio ($<70\%$) did not affect the number of cells expressing IL-17A in sinonasal tissues (serum total IgE levels, $P = .861$; blood eosinophil counts, $P = .621$; and FEV₁/FVC ratio, $P = .429$). These results suggest that although demographic differences in blood eosinophil counts, serum total IgE levels, and FEV₁/FVC ratios were seen among the 3 groups, these biases did not seem to affect the differences in the number of cells expressing IL-17A in sinonasal tissues among the groups.

The number of IL-17A⁺ cells was significantly and positively correlated with the degree of eosinophilia, but not neutrophilia, in the NPs. This result is consistent with the recently published report by Saito et al.^{E24} and suggests that the expression of IL-17A is associated with eosinophilic inflammation in patients with CRS, especially in Japan. Our results also show a significant and positive correlation between the total number of IL-17A⁺ cells and the radiologic severity of sinusitis, reflecting the evidence that the radiologic severity of sinusitis is correlated with the degree of eosinophilia in sinonasal tissue in patients with CRSwNPs.^{E25} This finding is also contrary to the report by Saito et al.,^{E24} in which no association was identified between the number of IL-17A⁺ cells and the radiologic severity of sinusitis. These differences might be due to the differences in sample selection. Our samples included various phenotypes, such as non-CRS, CRSwONPs, and CRSwNPs, whereas samples in the Saito et al study only included patients with CRSwNPs. In combination, our results suggest that the local expression of IL-17A is positively associated with mucosal inflammation in patients with CRS.

Previous studies reported that eosinophils and CD4⁺ T cells in NPs express IL-17A.^{E24,E26} Our results from the double-immunofluorescence staining experiments demonstrate that macrophages in NPs express IL-17A, suggesting that macrophages also participate in IL-17A production. Human alveolar

macrophages also express IL-17A. In addition, the percentage of IL-17A⁺ cells present in the alveolar macrophages of patients with allergic asthma has been reported to be much higher than in those from control subjects.^{E27} Lung macrophages, rather than T_H17 cells or other immune cell types, are possibly the main producers of IL-17A in murine models of allergic asthma.^{E27} Thus macrophages in DNPCs might be the main sources of SEB-induced IL-17A production.

It remains unclear as to how IL-17A drives eosinophilic inflammation. Thus we aimed to determine the direct effects of recombinant IL-17A on cytokine production by DNPCs. Recombinant IL-17A did not induce the synthesis of IL-5, IL-13, RANTES, or eotaxin. It is known that IL-17A is able to induce eotaxin expression in human airway smooth muscle; however, this is not the case in the upper airway.^{E20} In contrast, IL-17A was found to directly induce GM-CSF and IL-6 production by DNPCs. IL-17A is able to induce GM-CSF production in lung microvascular endothelial cells.^{E28} GM-CSF is expressed on the infiltrating inflammatory cells and epithelium in NPs.^{E29} Eosinophils also express the GM-CSF receptor alpha chain, and GM-CSF is involved in priming, chemotaxis, cytokine production, and inhibition of apoptosis in eosinophils.^{E30,E31} GM-CSF deficiency leads to impairment of eosinophil infiltration into the airways in murine models of asthma.^{E31} IL-6 production is also known to be induced by IL-17A stimulation in the bronchial fibroblasts of asthmatic patients.^{E32} IL-6 promotes T_H2 differentiation through the induction of nuclear factor of activated T cells and c-Maf during early CD4⁺ T-cell activation.^{E33} Furthermore, local disruption of IL-6 signaling in the lung decreases antigen-induced eosinophilic inflammation.^{E34} Our results might suggest that one of the mechanisms underlying the positive correlation between the local expression of IL-17A and eosinophilia in CRSwNPs is induction of GM-CSF and IL-6 production by IL-17A. However, because the change in GM-CSF and IL-6 produced by the addition of IL-17A is not very large, future studies should be performed examining whether the induction of GM-CSF and IL-6 has a biologically important effect on eosinophilia.

The present study provides evidence that IL-17A expression is associated with eosinophilic inflammation of CRSwNPs both *in vivo* and *ex vivo* and that the production of IL-17A by DNPCs in response to SEB is regulated by cytokines, including IFN- γ , IL-12/IL-23 p40, and PGE₂. EP2 and EP4 receptors also mediate the PGE₂-induced production of IL-17A, whereas the activation of the EP3 receptor displays an opposite effect. These observations might provide a basis for future therapeutic approaches targeting not only IL-17A but also regulatory cytokines, PGE₂, and EP receptor-selective agonists in the management of severe eosinophilic airway diseases, including CRSwNPs, allergic rhinitis, and bronchial asthma. On the other hand, our study has several limitations, including comparison of different tissues (ie, NPs vs nasal mucosa). In addition, measurement of IL-17A in nasal lavage samples and characterization of other members of the IL-17 family should be made in future investigations.

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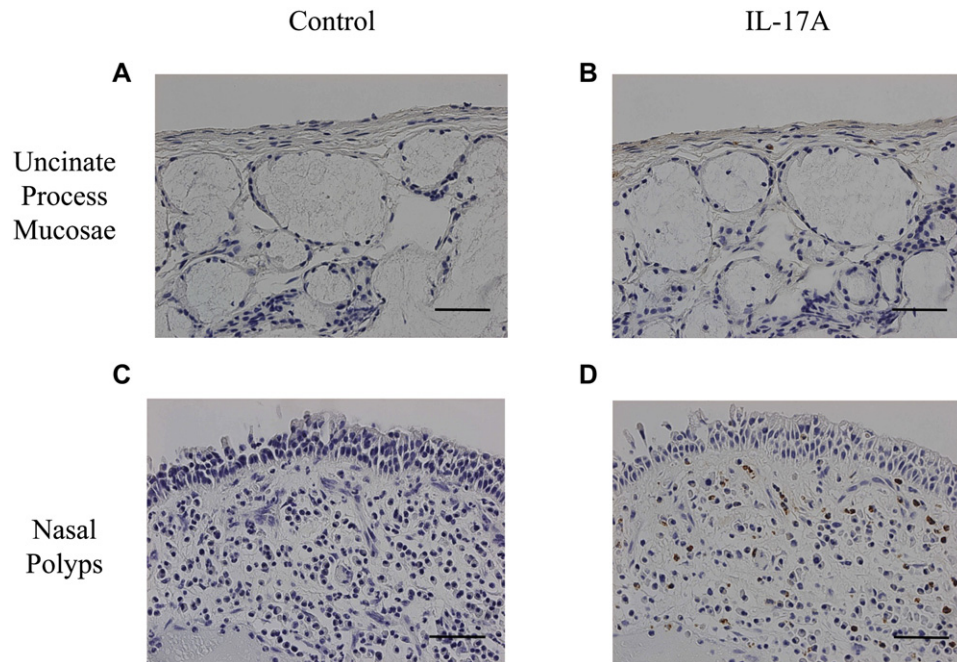


FIG E1. Immunohistochemical staining of IL-17A in UPM (**A** and **B**) and NPs (**C** and **D**) from a patient without CRS and a patient with aspirin-intolerant asthma. Sections were reacted with rabbit polyclonal antibody against IL-17A (**Fig E1, B** and **D**) or control (Universal Negative Control [rabbit]; **Fig E1, A** and **C**), after which they were stained with a Histofine MAX-PO with a diaminobenzidine substrate. Scale bar = 50 μ m.

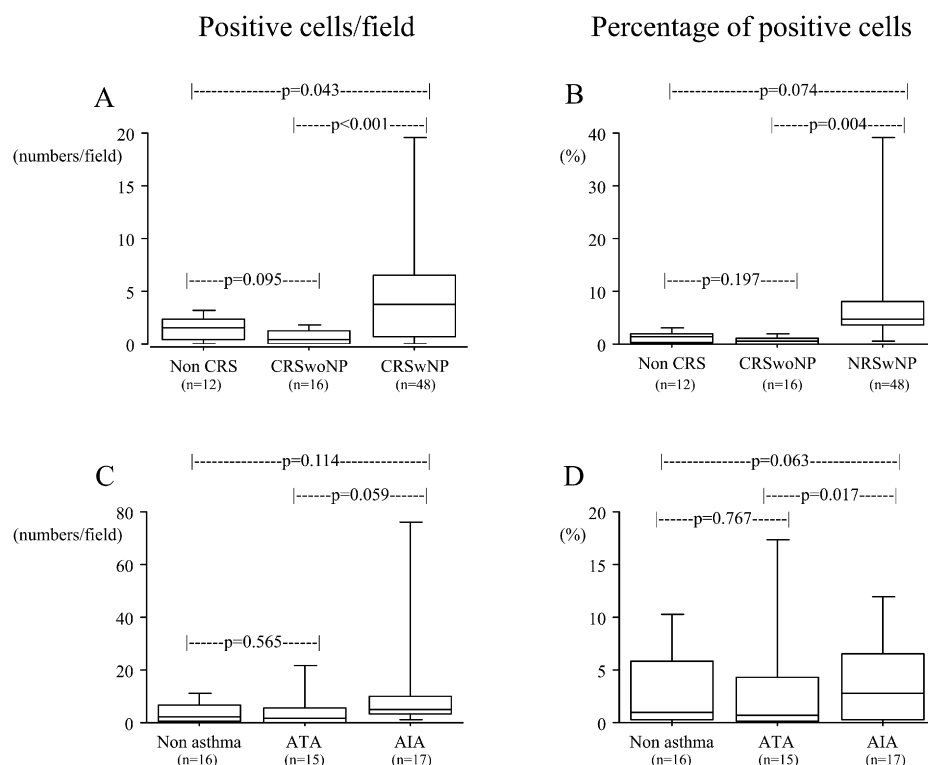


FIG E2. Comparison of numbers (A and C) and rates (B and D) of IL-17A⁺ cells among various phenotypes of CRS. Numbers of cells expressing IL-17A per field (Fig E2, A) and percentage of cells expressing IL-17A in infiltrating inflammatory cells (Fig E2, B) in NPs from patients with CRSwnNPs (n = 48) were compared with those in UPM from patients without CRS (n = 12) and patients with CRSwoNPs (n = 16). Numbers of cells expressing IL-17A per field (Fig E2, C) and percentage of cells expressing IL-17A in infiltrating inflammatory cells (Fig E2, D) in NPs were compared among patients without bronchial asthma (n = 16), with aspirin-tolerant asthma (ATA; n = 15), and with aspirin-intolerant asthma (AIA; n = 17). Rectangles include the range from the 25th to 75th percentiles, horizontal lines indicate the median, and vertical lines indicate the range from the 10th to 90th percentiles. P values were determined by using the Mann-Whitney U test.

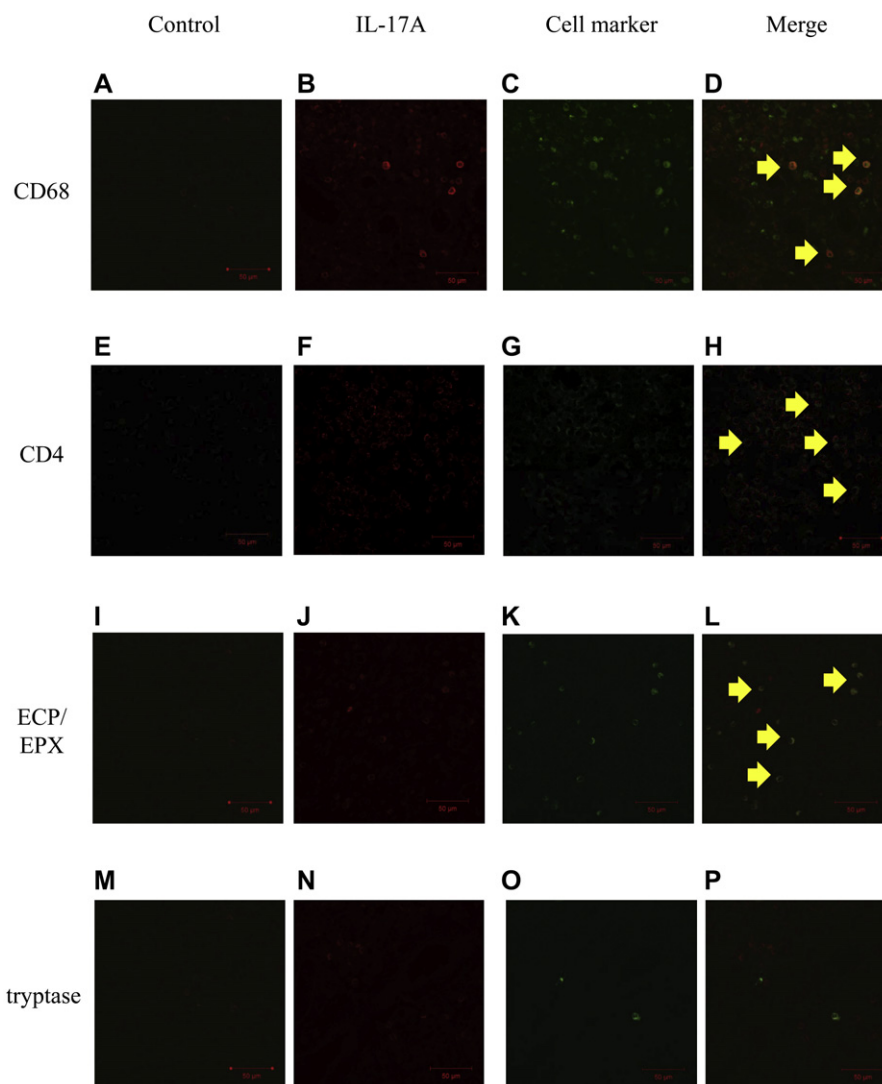


FIG E3. Expression of IL-17A in infiltrating CD68⁺ (D), CD4⁺ (H), eosinophil cationic protein/eosinophil protein X (ECP/EPX)⁺ (L), and tryptase-positive (P) cells in NPs. As controls, sections were stained with rabbit anti-IL-17 polyclonal antibody alone (B, F, J, and N); murine anti-CD68 (C), CD4 (G), eosinophil cationic protein/eosinophil protein X (ECP/EPX) (K), and tryptase (O) mAbs alone; or control antibodies (Universal Negative Control [rabbit] and [mouse]). Arrows indicate double-immunostained cells. Scale bar = 50 μm.

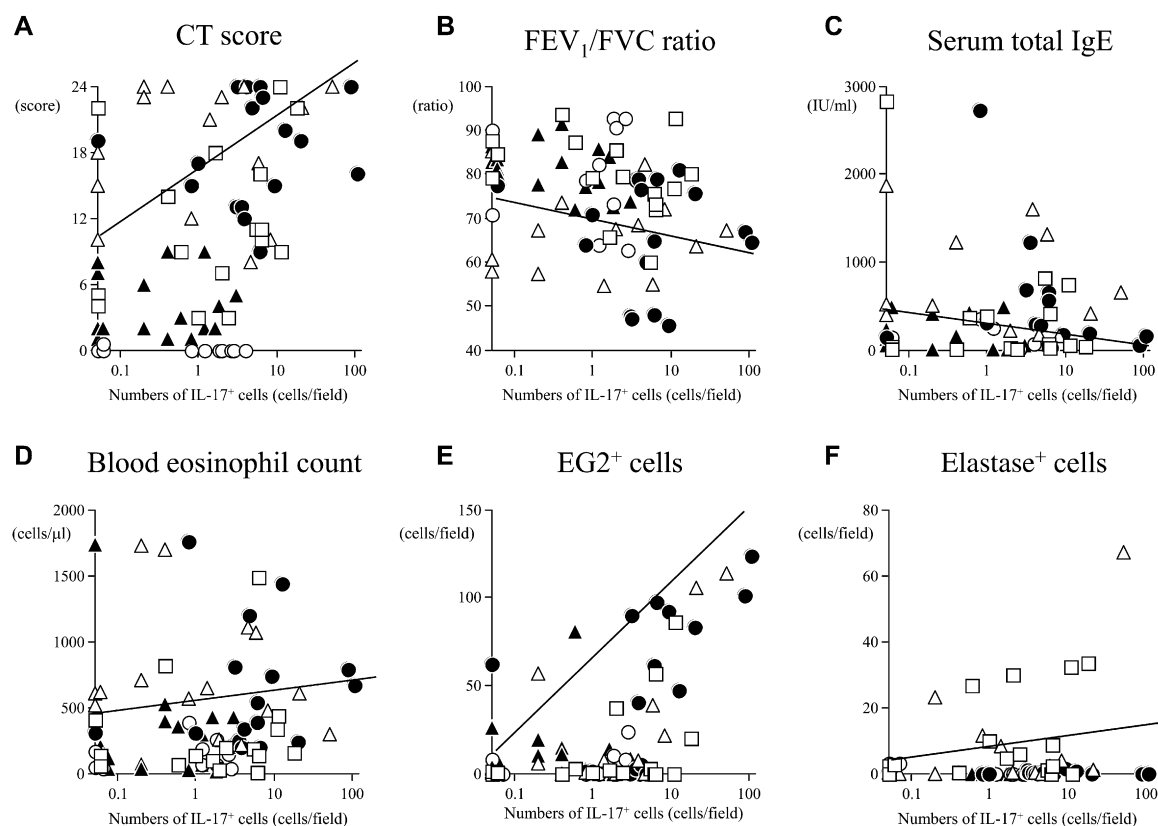


FIG E4. Relationship between numbers of IL-17A⁺ cells in sinonasal tissues and pathophysiological characterizations, including radiologic severity of sinusitis (**A**), FEV₁/FVC ratio (**B**), serum total IgE level (**C**), blood eosinophil count (**D**), number of infiltrating Eosinophil cationic protein/eosinophil protein X (ECP/EPX)⁺ cells in sinonasal tissues (**E**), and number of elastase-positive cells in sinonasal tissues (**F**). *Open circles, closed triangles, open squares, open triangles, and closed circles* represent patients without CRS, patients with CRSwNPs, patients with CRSwNPs without asthma, patients with CRSwNPs with aspirin-tolerant asthma, and patients with CRSwNPs with aspirin-intolerant asthma, respectively. *CT*, Computed tomography.

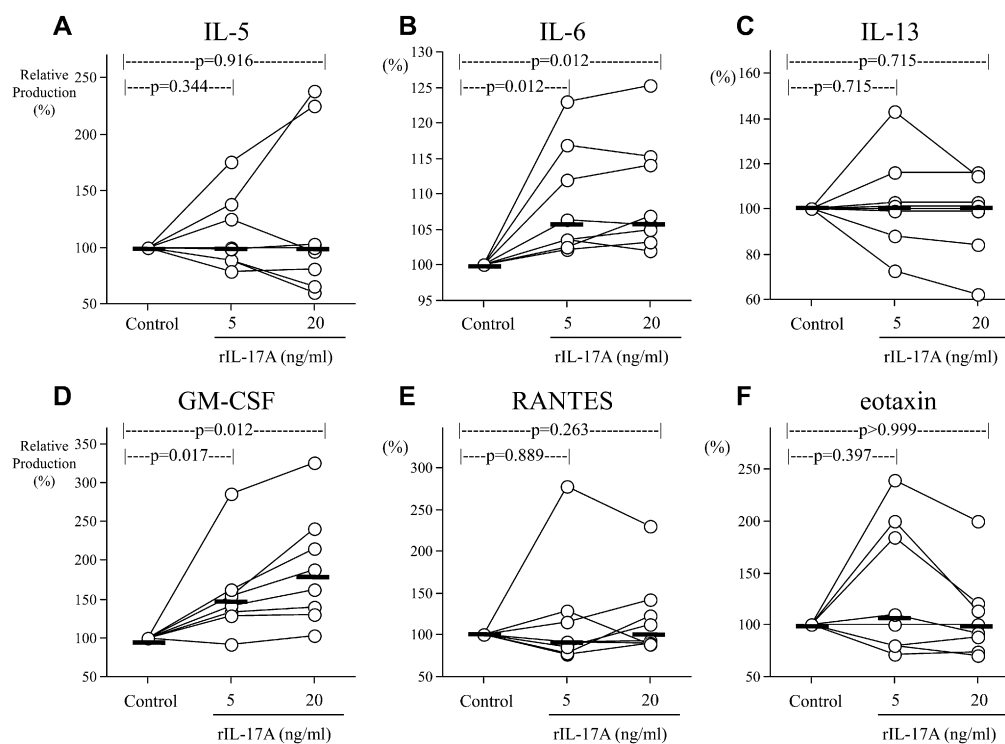


FIG E5. Direct effect of recombinant IL-17A on cytokine production by DNPCs. DNPCs ($n = 8$) were incubated with 5 and 20 ng/mL recombinant IL-17A or control buffer (800 nmol/L HCl). After a 72-hour incubation, culture supernatants were collected, and the levels of IL-5 (**A**), IL-6 (**B**), IL-13 (**C**), GM-CSF (**D**), RANTES (**E**), and eotaxin (**F**) were measured by means of ELISA. Results were shown as a relative production. P values were determined by using the Wilcoxon signed-rank test.

TABLE E1. Subjects' characteristics

	Patients without CRS	Patients with CRS without NPs	Patients with CRS with NPs without BA	Patients with CRS with NPs with ATA	Patients with CRS with NPs with AIA
No.	12	16	16	15	17
Sex (male/female)	4/8	8/8	12/4	10/5	9/8
Age (y)	51.1 ± 18.4	52.1 ± 17.1	43.4 ± 18.0	56.0 ± 13.4	50.9 ± 14.5
Age range (y)	15-81	14-74	14-73	21-77	22-77
Serum IgE (IU/mL)	164 ± 140	193 ± 184	390 ± 729	754 ± 589	533 ± 710
Blood eosinophil count ($\times 10^2/\mu\text{L}$)	1.36 ± 1.09	3.04 ± 4.18	2.92 ± 3.78	7.39 ± 4.66	6.08 ± 4.68
CT grading score	0	4.0 ± 2.9	11.8 ± 6.9	18.3 ± 5.9	18.2 ± 4.9
FEV ₁ /FVC ratio	79.4 ± 11.2	81.0 ± 5.7	79.5 ± 9.1	66.5 ± 9.4	66.2 ± 12.6

AIA, Aspirin-induced asthma; ATA, aspirin-tolerant asthma; BA, bronchial asthma; CT, computed tomography.

TABLE E2. Anti -human antibodies used for immunohistochemistry

Cell type	Molecule	Clone	Isotype	Dilution	Manufacturer
Mast cell	c-kit	AAI	Murine IgG1	1:100	MBL, Nagoya, Japan
Eosinophil	ECP/EPX	EG2	Murine IgG1	1:100	Pharmacia, Uppsala, Sweden
Neutrophil	Elastase	NP57	Murine IgG1	1:100	Dako Japan, Tokyo, Japan
B cell	CD79 α	JCB117	Murine IgG1	1:50	Dako Japan
Macrophage	CD68	KP1	Murine IgG1	1:50	Dako Japan
T cell	CD4	4B12	Murine IgG1	1:50	MBL
	CD8	1A5	Murine IgG1	1:50	MBL
Epithelial cell	Cytokeratin	AE1/AE3	Murine IgG1	1:50	Dako Japan
Vascular endothelial cell/fibroblast	Vimentin	V9	Murine IgG1	1:50	Dako Japan

ECP/EPX, Eosinophil cationic protein/eosinophil protein X.