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**Group 2 innate lymphoid cells are increased in nasal polyps in patients with
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Short title: Group 2 innate lymphoid cells in chronic rhinosinusitis

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Abstract

ILC2s represent a critical innate cellular source of type 2 cytokines and may play important roles in various diseases. We examined the role of ILC2s in the pathogenesis of two subgroups of CRSwNP; ECRS and non-ECRS. We analyzed the prevalence of ILC2s in sinonasal tissues and in blood from patients with ECRS, non-ECRS, CRSsNP and control. The prevalence of ILC2s in nasal tissues was higher in patients with ECRS as compared to those with non-ECRS or CRSsNP. The prevalence of blood ILC2s was not different between patients with ECRS and non-ECRS. The prevalence of blood ILC2s was higher in patients with allergic rhinitis and elevated serum IgE levels. *Alternaria*-induced IL-33 secretion was increased in nasal epithelial cells derived from patients with ECRS as compared to those from patients with non-ECRS or CRSsNP. ILC2s may be involved in the pathogenesis of CRSwNP, in particular in patients with tissue eosinophilia (i.e. ECRS).

Key words: Group 2 innate lymphoid cells, eosinophilic chronic rhinosinusitis, IL-33, allergic rhinitis, asthma, *Alternaria*

Abbreviations: ILC2s, group 2 innate lymphoid cells; CRS, chronic rhinosinusitis; CRSwNP, chronic rhinosinusitis with nasal polyps; CRSsNP, chronic rhinosinusitis without nasal polyps; ECRS, eosinophilic chronic rhinosinusitis; UTs, uncinat tissues; NPs, nasal polyps; AR, allergic rhinitis; FACS, fluorescence activated cell sorting, PBMCs, peripheral blood mononuclear cells;

1. Introduction

Innate lymphoid cells (ILCs) are emerging as important effector cells of the innate immune system, which may play important roles in pathogen clearance, tissue remodeling and immune homeostasis. Among ILCs, group 2 innate lymphoid cells (ILC2s) are a recently identified subset that produces large amounts of type 2 cytokines, such as IL-5 and IL-13. Epithelium-derived IL-33, IL-25 and thymic stromal lymphopoietin (TSLP) are likely to be the key cytokines for induction of the production of type 2 cytokines by ILC2s [1]. Recent studies suggest that ILC2s may play important roles in innate allergic inflammation [2-4].

Chronic rhinosinusitis (CRS) is characterized by persistent symptomatic inflammation of the nasal and paranasal mucosa that lasts longer than 12 weeks [5]. CRS can be further classified into 2 major subtypes: CRS without nasal polyps (CRSsNP) and CRS with nasal polyps (CRSwNP) [5]. In the US and in European countries, CRSsNP presents as a predominant infiltration of neutrophils and Th1- or Th17-type cytokines, whereas CRSwNP is characterized by eosinophilic infiltration and Th2-type cytokines. However, in Asian countries, CRSwNP may represent both eosinophilic and

neutrophilic phenotypes [6, 7]. Recently, the Japanese Epidemiological Survey of Refractory Eosinophilic Chronic Rhinosinusitis Study (JESREC Study) classified CRSwNP into two subtypes; eosinophilic CRS (ECRS) and non-ECRS. ECRS represents marked eosinophilia in nasal polyps and is associated with greater clinical and radiological severity, high morbidity with bronchial asthma, and higher risk of polyp recurrence, as compared to non-ECRS [8].

In our previous study, IL-33, IL-25 and TSLP were detected at higher levels in epithelial cells of nasal polyps from ECRS patients as compared to those from non-ECRS patients [9]. Human ILC2s were first identified in nasal polyp tissues, and increased numbers of ILC2s were found in nasal polyps [3]. However, the functional importance of ILC2s in the pathogenesis of CRS is not clearly understood. In addition, only a few reports examined the ILC2s in peripheral blood in association with allergic diseases [10, 11]. To fill this gap in our knowledge, in the present study, we analyzed the prevalence of ILC2s in sinonasal mucosa and nasal polyps and in peripheral blood from patients with ECRS, as compared to patients with non-ECRS; patients with CRSsNP and those without CRS were also used as controls. Our findings suggest that ILC2s are increased in nasal polyps of patients with ECRS as compared to those with non-ECRS. The increased prevalence of blood ILC2s was associated with the presence

of allergic rhinitis and elevated serum IgE levels. Furthermore, airway epithelial cells from patients with ECRS produced larger quantities of IL-33 when stimulated *in vitro* with allergen as compared to those produced from non-ECRS.

2. Materials and methods

2.1. Study subjects

Patients with sinonasal diseases were recruited from the department of Otorhinolaryngology at Shiga University of Medical Science hospital according to the protocol approved by the Institutional Review Board of Shiga University of Medical Science (ethics approval number 25-36). Informed consent was obtained from all participants. Sinonasal tissues of CRS patients such as uncinate tissues (UTs) and nasal polyps (NPs) were obtained during endoscopic sinus surgery, and peripheral blood samples were collected preoperatively. The diagnosis of CRS was made based on clinical, endoscopic and radiographic criteria as described by Fokkens et al.[5] in the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) 2012. Control subjects who did not have CRS but did have asthma, allergic rhinitis (AR) or hypertrophic rhinitis were also recruited. A detailed list of the patients' characteristics is presented in Table 1. Patients with CRS with nasal polyps (CRSwNP) were divided into ECRS and non-ECR according to a published clinical scoring system (JESREC score),

and mucosal eosinophilia that was defined as higher than 70 per high-power field (HPF) [8]. The JESREC score consists of bilateral disease sites (3 points), nasal polyps (2 points), CT shadow: ethmoid > maxillary sinus (2 points), and blood eosinophilia: 2-5% (4 points), 5-10% (8 points), or more than 10% (10 points), and patients with scores higher than 11 points were defined as ECRS. All CRS patients had previously failed conservative medical treatments and saline irrigations. The patients were not treated with systemic corticosteroids within 4 weeks before the surgery. Patients with respiratory epithelial adenomatoid hamartoma (REAH), post-operative sinus cyst, fungal sinusitis, or CRS with sinonasal tumor were excluded from the study. The diagnosis of asthma was based on criteria of the Global Initiative for Asthma, published in 2006 [12]. Allergic rhinitis (AR) was diagnosed based on “Practical Guideline for the Management of Allergic Rhinitis in Japan,” published in 2009 [13].

Patients were assigned to four groups: ECRS (n=14), non-ECRS (n=16), CRSsNP (n=16) and control subjects (n=17: 13 patients with allergic rhinitis with or without asthma, 4 patients with hypertrophic rhinitis) (Table 1). The percentage of peripheral blood eosinophils was significantly higher in ECRS patients ($8.9 \pm 0.8\%$), compared with non-ECRS patients ($5.7 \pm 1.3\%$), CRSsNP patients ($4.1 \pm 0.7\%$), or control subjects ($5.3 \pm 1.2\%$).

2.2. Isolation of ILC2s from peripheral blood and sinonasal tissues

Heparinized peripheral blood was layered over an equal volume of Histopaque 1083 (Sigma-Aldrich; St Louis, Mo), and centrifuged according to the manufacturer's instructions. Peripheral blood mononuclear cells (PBMCs) were collected from the interface between Histopaque and plasma. PBMCs were washed and resuspended in RPMI 1640 media (Wako; Osaka, Japan) containing 10% fetal bovine serum (FBS) (bioewest; Nuaille, France), penicillin (100 U/mL), and streptomycin (100 µg/mL) (Wako).

Sinonasal tissues were cut into fine pieces and digested for 30-45 min at 37 °C with Liberase TM (125 µg/mL) and DNase I (200 µg/mL) (both from Roch Diagnostics GmbH; Mannheim, Germany). Alternatively, cells were isolated by mechanical disruption of the tissues with the gentleMACS Dissociator and Tumor Dissociation Kit (Miltenyi Biotec Inc.; Auburn, CA) following the procedure recommended by the manufacturers. The cell suspensions were filtered through a 70 µm nylon mesh. The remaining cells were treated with ACK lysing buffer (Lonza) to lyse red blood cells.

Fluorescence activated cell sorting (FACS) was used to identify ILC2s following

the strategy used by Mjösberg et al [3]. Briefly, PBMCs or total cell suspensions from sinonasal tissues were stained with the following antibody cocktail: Fluorescein isothiocyanate (FITC)-labeled antibodies to CD3, CD14, CD16, CD19, CD20, CD56 (Lineage cocktail), phycoerythrin (PE)-cyanine 7 (Cy7)-labeled antibody to CD45, PE-labeled antibody to CD127, and Alexa Fluor[®]-labeled antibody to CRTH2 (BioLegend; San Diego, CA). ILC2s were identified as lineage⁻ CD45⁺ CD127⁺ CRTH2⁺ cells. The prevalence of ILC2s was calculated as the numbers of ILC2s divided by the numbers of total lineage⁻ CD45⁺ cells.

2.3. ILC2s culture

In some experiments, ILC2s were sorted from sinonasal and peripheral blood specimens. Sorted ILC2s (1.0×10^3 cells/mL) were cultured in 96-well round-bottom plates in RPMI 1640 containing 10% autologous serum at 37 °C with 5% CO₂ for 8 days with or without IL-33 (10 ng/mL, R&D System; Minneapolis, MN) in the presence or absence of IL-2 (50 ng/mL, R&D System). The concentrations of IL-5 and IL-13 in cell-free supernatants were determined using ELISA kits (R&D System).

2.4. Epithelial Cell culture

Nasal epithelial cells were obtained by scratching UTs of patients with CRSsNP or NPs of patients with ECRS or non-ECRS by using curettes (ASI; Springville, UT). Cells were seeded in supplemented basal epithelial growth medium (Lonza Corp.; Walkersville, MD) and were cultured in a type I collagen-coated 75-cm² flask (Iwaki; Tokyo, Japan) in a humidified incubator at 37 °C with 5% CO₂. Once confluent, nasal epithelial cells were subcultured and seeded onto a type I collagen-coated 24-well tissue culture plate (Iwaki). After the cells were grown to confluency, *Alternaria* extract (100 µg/mL, Greer Laboratories; Lenoir, NC) was added, and the cells were incubated for 2 hours. Cell-free supernatants were collected and stored at -20 °C until use. The concentration of IL-33 was determined using an ELISA kit (R&D System).

2.5. Statistical analyses

Data are presented as means ± SEM for the number of subjects or experiments as indicated. Statistical analysis was performed using the Mann-Whitney *U* test. Correlations were assessed by using Spearman's rank correlation. *P* < 0.05 was

considered statistically significant.

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3. Results

3.1. ILC2s in peripheral blood and in nasal polyps

ILC2s were identified as lineage negative (Lin^-) CD45^+ CD127^+ CRTH2^+ cells in PBMCs (Fig. 1A) and in nasal polyps (Fig. 1B). To examine the ability of ILC2s to produce cytokines, ILC2s and control cell populations (i.e. Lin^- CD45^+ CD127^- CRTH2^- and Lin^- CD45^+ CD127^+ CRTH2^-) were stimulated with IL-33 (10 ng/mL) and IL-2 (50 ng/mL), and IL-5 production was measured using ELISA. ILC2s but not the other cell populations tested produced a large quantity of IL-5 (Fig. 1C). The number of Lin^- CD45^+ CD127^- CRTH2^+ cells was too small to be examined.

To further characterize the responses of ILC2s to cytokines, ILC2s were cultured for 8 days with or without IL-33 (10 ng/mL) and/or IL-2 (50 ng/mL). ILC2s from PBMCs produced IL-5 in response to IL-33 or IL-2. IL-2 alone, but not IL-33 alone, induced IL-13 production. Nonetheless, a combination of IL-33 and IL-2 robustly induced IL-5 and IL-13 production (Fig. 2A). ILC2s from nasal polyp (NP) tissues produced both IL-5 and IL-13 when stimulated with IL-2 alone but not when stimulated with IL-33 alone.

Similar to the PBMC-derived ILC2s, NP-derived ILC2s produced high and roughly comparable amounts of IL-5 and IL-13 when they were stimulated with a combination of IL-33 and IL-2 (Fig. 2B). These results indicate that PBMC-derived and NP-derived ILC2s have a similar ability to produce IL-5 and IL-13.

We then examined the cell numbers of ILC2s after 8 days in culture. IL-2 or IL-33 plus IL-2 significantly increased the number of ILC2s from both PBMCs (Fig. 2C) and NPs (data not shown).

3.2. The prevalence of ILC2s in nasal polyps

To investigate the roles of ILC2s in CRSwNP, we compared the prevalence of ILC2s in NPs from ECRS and non-ECRS patients. The prevalence of ILC2s in NPs was significantly higher in ECRS patients, compared to non-ECRS patients (Fig. 3A). In addition, the prevalence of ILC2s in NPs from ECRS patients was significantly higher than that in UTs from patients with CRSsNP. The prevalence of ILC2s showed a positive correlation with the number of infiltrating eosinophils in NPs (Fig. 3B).

However, the prevalence of ILC2s in NPs was not correlated with the percentage of blood eosinophils (Fig. 3C), with the severity of disease as evaluated with the JESREC

score (Fig. 3D) or with CT findings as indicated by the Lund-Mackay score (Fig. 3E).

These combined findings suggest that ILC2s are increased in nasal polyp tissues of CRSwNP patients, in particular in those with ECRS or tissue eosinophilia.

3.3. The prevalence of ILC2s in peripheral blood

To examine whether these observations in the local tissues are reproduced in systemic circulation, we analyzed eosinophils and ILC2s in peripheral blood.

The percentage of blood eosinophils was significantly higher in ECRS as compared to non-ECRS, CRSsNP, or control subjects (Fig. 4A). In contrast, there was no difference in the prevalence of ILC2s in PBMCs between ECRS and non-ECRS.

Furthermore, the prevalence of ILC2s was significantly lower in ECRS as compared to CRSsNP (Fig. 4B). The prevalence of ILC2s was not correlated with disease severity evaluated by JESREC score (Fig. 4C) or Lund-Mackay score (Fig. 4D).

The prevalence of ILC2s in PBMCs was significantly low in patients with blood eosinophilia (e.g. >10%) as compared to those with less eosinophils (<5%) (Fig. 5A). Interestingly, ILC2s in peripheral blood were increased in patients with high total serum IgE levels (i.e. >400 IU/mL) compared to those with lower IgE levels, although this

increase was statistically insignificant. (Fig. 5B). Among the patients without asthma, the prevalence of ILC2s was significantly higher in those with AR as compared to those without AR (Fig. 5C), and total serum IgE level was significantly higher in those with AR (264 ± 49 IU/mL) as compared to those without AR (104 ± 23 IU/mL, $P < 0.05$). On the other hand, the prevalence of ILC2s was significantly lower in those with asthma than in those without asthma (Fig. 5D) while the percentage of blood eosinophils was significantly higher in patients with asthma (8.4 ± 1.0 %) as compared to patients without asthma (5.0 ± 0.6 %, $P < 0.01$).

3.4. *Alternaria*-induced release of IL-33 from cultured nasal epithelial cells

These findings led us to speculate that airway sensitivity to allergen exposure may be involved in the increased prevalence of tissue ILC2s in patients with ECRS. IL-33 is an important cytokine that induces proliferation and activation of ILC2s [1, 14, 15]. We therefore examined the ability of airway epithelial cells to produce IL-33 in response to allergen exposure. To this end, we stimulated nasal airway epithelial cells with a fungal allergen, *Alternaria*, and examined the amounts of IL-33 in cell-free supernatants. Nasal epithelial cells from NPs of ECRS patients (n=16) released significantly higher amounts

of IL-33 as compared with the amounts released from NPs of non-ECRS patients (n=6) and from UTs of CRSsNP patients (n=12). *Alternaria* did not induce significant IL-33 release from nasal epithelial cells from the NPs of non-ECRS patients or from UTs of CRSwNP patients (Fig. 6). These results indicate that nasal epithelial cells from patients with ECRS readily release IL-33 upon allergen exposure, which may explain the increased prevalence of ILC2s in the NP tissues of those patients.

4. Discussion

Recent advances in our understanding of epithelial cell-derived cytokines and ILC2s indicate their critical roles in Th2 type immune responses of airway inflammation. IL-33, IL-25 and TSLP are released by airway epithelial cells, and ILC2s that respond to these cytokines quickly produce a large quantity of Th2 cytokines and growth factors such as IL-5, IL-9, IL-13 and amphiregulin, resulting in airway eosinophilia, mucus production and tissue remodeling. Human ILC2s were first isolated from nasal polyps (NPs), lungs and gut, and NPs were shown to contain higher proportions of ILC2s when compared with uninflamed nasal mucosa of healthy controls [3]. CRS is a heterogeneous disease with different phenotypes. CRSsNP is usually characterized by neutrophil/lymphocyte infiltration and Th1-dominant inflammation, while the immunological phenotypes of CRSwNP are Th2-dominant with eosinophil infiltration [16]. However, this classification of CRS may not apply in Japan and other countries in East Asia, because half of the CRSwNP patients in these countries do not show eosinophil-predominant inflammation [6, 17, 18]. Therefore, In Japan, the term “eosinophilic chronic rhinosinusitis (ECRS)” was introduced to identify the subgroup of

CRSwNP, which shows distinct tissue eosinophilia as compared to CRSwNP without eosinophilia (i.e. non-ECRS).

In the present study, we found that the prevalence of ILC2s in NPs was significantly higher in ECRS patients compared with non-ECRS patients, and it was positively correlated with the numbers of infiltrating eosinophils in NPs. These results are consistent with previous studies, in which increased numbers of ILC2s were found in NPs from CRSwNP patients compared with in the nasal mucosa from CRSsNP patients or healthy controls [19, 20], and increased prevalence of ILC2s was correlated with tissue eosinophilia [21]. This study is the first report to compare the prevalence of ILC2s in NPs between ECRS and non-ECRS patients who were diagnosed according to the JESREC study [8]. We also found that a common airborne allergen, *Alternaria*, significantly stimulated the release of IL-33 from cultured nasal epithelial cells from ECRS patients compared with IL-33 release from non-ECRS patients and other control subjects. In our previous study, IL-33 protein was significantly elevated in cell lysates of the epithelial cells of NPs from ECRS patients compared to those from non-ECRS patients [9]. These results suggest the critical involvement of IL-33 and ILC2s in the pathophysiology of ECRS. Increased production of IL-33 by airway epithelial cells may promote the proliferation of, and IL-5 and IL-13 release from ILC2s, resulting in tissue

eosinophilia, mucus production and formation of NPs that are distinctively observed in ECRS.

The present study demonstrated the prevalence of ILC2s in PBMCs in association with different conditions of the patients, including bronchial asthma and allergic rhinitis. The percentage of blood eosinophils was significantly higher in ECRS patients as compared to non-ECRS patients; however, the prevalence of ILC2s in PBMCs was not increased in ECRS patients. The prevalence of ILC2s in PBMCs was significantly lower in patients with bronchial asthma and in those with higher blood eosinophilia. On the other hand, increased prevalence of ILC2s in PBMCs was observed in patients with allergic rhinitis and with higher serum IgE levels.

ILC2s are recruited directly to the nasal mucosa through the peripheral blood circulation [3]. In a previous human study, the numbers of cytokine-producing ILC2s in PBMCs from allergic asthma patients were greater than the numbers in AR patients and healthy controls [10]. Peripheral blood ILC2s were increased during the pollen season in patients with grass pollen allergic rhinitis, and this increase was inhibited by subcutaneous grass pollen immunotherapy [22]. Increased prevalence of ILC2s in PBMCs was also demonstrated in AR patients 4 hours after nasal cat allergen exposure [23]. These results are consistent with the results of our present study, in which the

prevalence of ILC2s in PBMC was increased in AR patients with high serum IgE levels.

Thus, increased prevalence of ILC2s in PBMCs may reflect type I allergic immune responses.

The prevalence of ILC2s in NPs was positively correlated with the numbers of infiltrating eosinophils; however, it was significantly lower in the PBMCs of patients with higher blood eosinophilia. The reason for this discrepancy is not well understood. It is possible that preoperative medical treatment, such as inhaled corticosteroid therapy, may affect the prevalence of ILC2s in PBMCs, although the patients did not take systemic corticosteroids within 4 weeks before the surgery. Alternatively, ILC2s in peripheral blood may be recruited quickly to the tissues as soon as they exit from the bone marrow in patients with marked tissues eosinophilia (and presumably increased amounts of the tissues factors that recruit ILC2s from the circulation). The roles and functions of ILC2s in peripheral blood may be different from those in NPs, and further studies are required to determine the conditions that affect the prevalence of ILC2s in the peripheral circulation.

5. Conclusions

We have demonstrated the enrichment of ILC2s in NPs of ECRS patients and a positive correlation between the prevalence of ILC2s and the number of infiltrating eosinophils. We also demonstrated the increased release of IL-33 from cultured airway epithelial cells of ECRS patients in response to *Alternaria* extract. IL-33 stimulates the ILC2s to produce a large quantity of Th2 cytokines such as IL-5 and IL-13, resulting in tissue eosinophilia and mucus production. ILC2s and IL-33 may play a key role in the pathophysiology of ECRS, and thus are potential therapeutic targets for the treatment of ECRS.

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Conflict of interest

The authors declare that they have no conflicts of interest. The authors have no financial disclosures.

Figure legends

Table 1. Clinicopathological characteristics of patients with CRSsNP and of ECRS and non-ECRS subgroups of CRSwNP.

AIA, Aspirin-induced asthma

Fig. 1.

Fluorescence activated cell sorting (FACS) for determination of ILC2s. Representative data from a patient with ECRS are shown. (A) Peripheral blood mononuclear cell (PBMC)-derived ILC2s, (B) nasal polyp (NP)-derived ILC2s. Samples were initially gated on the lymphocyte population. The cells were then gated on lineage (Lin)⁻ and CD45⁺. Lin⁻ CD45⁺ lymphocytic cells were subdivided into 4 populations on the basis of CD127 and CRTH2 expression. (C) Sorted cell populations from PBMCs were stimulated for 5 days with IL-33 (10 ng/mL) and IL-2 (50 ng/mL) and IL-5 production was measured using ELISA. ILC2s were determined as Lin⁻ CD45⁺ CD127⁺ CRTH2⁺ cells, and only these cells could produce IL-5 in response to IL-33 and IL-2. Data shown are means ± SEM. * *P* <0.05; ***P* <0.01. *FSC*: Forward scatter; *SSC*: side scatter.

Fig. 2.

ILC2s derived from PBMC or NP produced high amounts of IL-5 and IL-13 and proliferated when they were stimulated with IL-33 and IL-2. ILC2s sorted from a patient with ECRS were cultured for 8 days with or without IL-33 (10 ng/mL) and/or IL-2 (50 ng/mL). Subsequently, IL-5 or IL-13 production was measured by ELISA in PBMC-derived (A) or NP-derived (B) ILC2s, and the cell number of PBMC-derived ILC2 cells was counted (c). (A) PBMC-derived ILC2s released IL-5 and IL-13 in response to IL-33 and IL-2. (B) NP-derived ILC2s released IL-5 and IL-13 in response to IL-33 and IL-2. (C) Proliferation of PBMC-derived ILC2s was observed in response to IL-33 and IL-2. Data shown are means $\pm$ SEM. * $P < 0.05$; ** $P < 0.01$.

Fig. 3.

The prevalence of ILC2s in NPs of ECRS patients was increased and positively correlated with the number of infiltrating eosinophils. The prevalence of ILC2s was calculated as ILC2s divided by $\text{Lin}^- \text{CD45}^+$ cells. (A) The prevalence of ILC2s was significantly higher in NPs from ECRS patients, compared with the prevalence in NPs from non-ECRS patients and in uncinate tissues (UTs) from CRSsNP patients. * $P < 0.05$; ** $P < 0.01$. (B) The prevalence of ILC2s was positively correlated with the

number of eosinophils in NPs (Pearson correlation coefficient, $R = 0.60$, $P < 0.01$). The prevalence of ILC2s in NPs was not correlated with the percentage of blood eosinophils (C), with the severity of disease as evaluated by the JESREC score (D) or with CT findings as assessed with the Lund-Mackay score (E).

Fig. 4.

The prevalence of ILC2s in peripheral blood of ECRS patients was not increased. (A) The percentage of blood eosinophils was significantly higher in ECRS patients compared with non-ECRS patients, CRSsNP patients and control subjects. * $P < 0.05$; ** $P < 0.01$. (B) The prevalence of ILC2s in PBMCs was lower in patients with ECRS than in CRSsNP patients. * $P < 0.05$. The prevalence of ILC2s in PBMCs was not correlated with disease severity evaluated by the JESREC score (C) or the Lund-Mackay score (D).

Fig. 5.

The prevalence of ILC2s in peripheral blood was low in patients with bronchial asthma and in those with higher blood eosinophilia, whereas it was high in patients with allergic rhinitis (AR). (A) The prevalence of ILC2s in PBMCs was significantly lower at higher

blood eosinophilia (more than 10% or 5-10% eosinophils), compared with at low blood eosinophilia (less than 5% eosinophils). * $P < 0.05$; ** $P < 0.01$. (B) The prevalence of ILC2s in PBMCs was relatively higher at a high serum IgE level of more than 400 IU/mL, compared with at a lower level of less than 200 IU/mL, although the difference was statistically insignificant. (C) In cases without asthma, the prevalence of ILC2s in PBMCs was significantly higher in patients with AR than in those without AR. * $P < 0.05$. (D) The prevalence of ILC2s in PBMCs was significantly lower in patients with bronchial asthma than in those without asthma. * $P < 0.05$.

Fig. 6.

Alternaria-induced IL-33 secretion was significantly increased in cultured nasal epithelial cells from patients with ECRS as compared to those from patients with non-ECRS or CRSsNP. *Alternaria* extract (100 $\mu\text{g/mL}$) stimulated IL-33 release from cultured nasal epithelial cells from the NPs of ECRS patients ($n=16$), whereas it did not stimulate IL-33 release from cultured nasal epithelial cells from the NPs of non-ECRS patients ($n=6$) or from the NPs from UTs of CRSsNP patients ($n=12$). Data shown are means $\pm$ SEM. ** $P < 0.01$.

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Figure 1

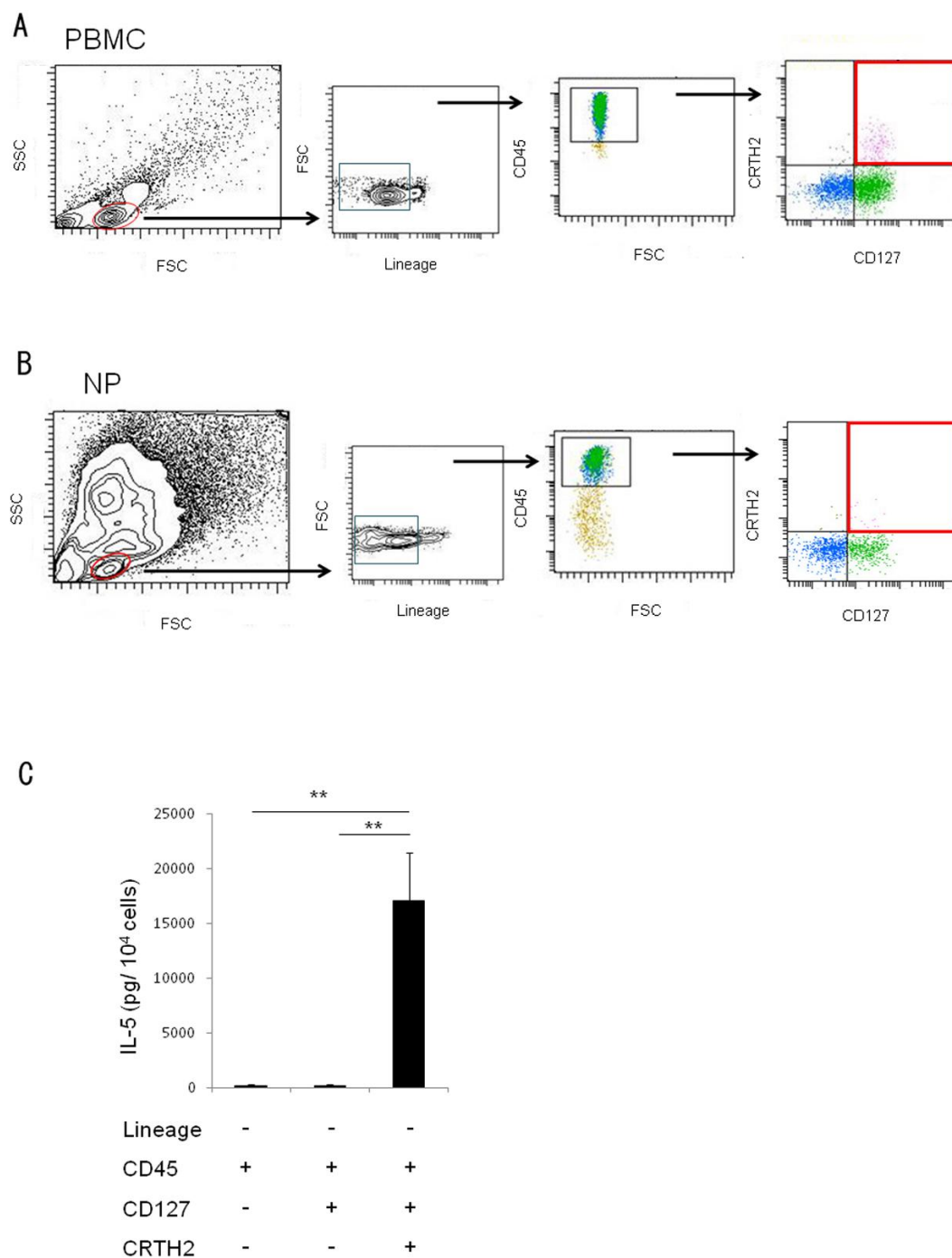
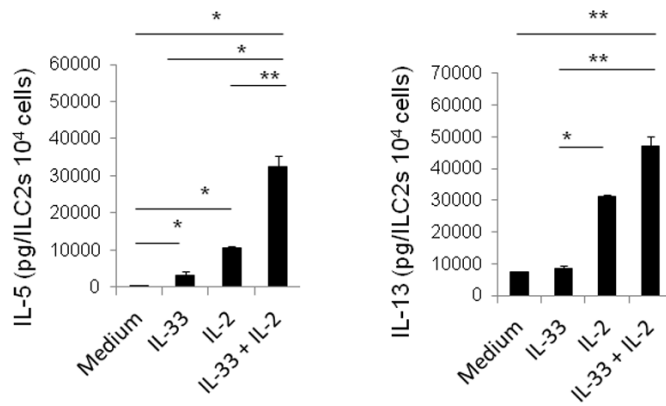
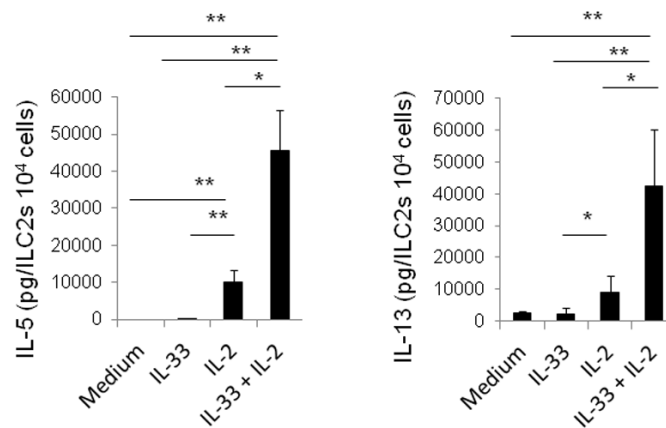


Figure 2

A



B



C

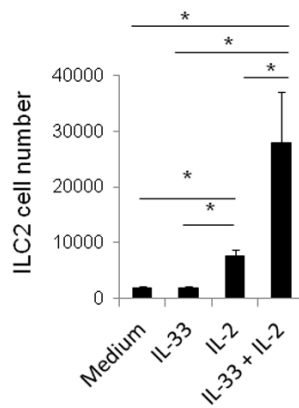


Figure 3

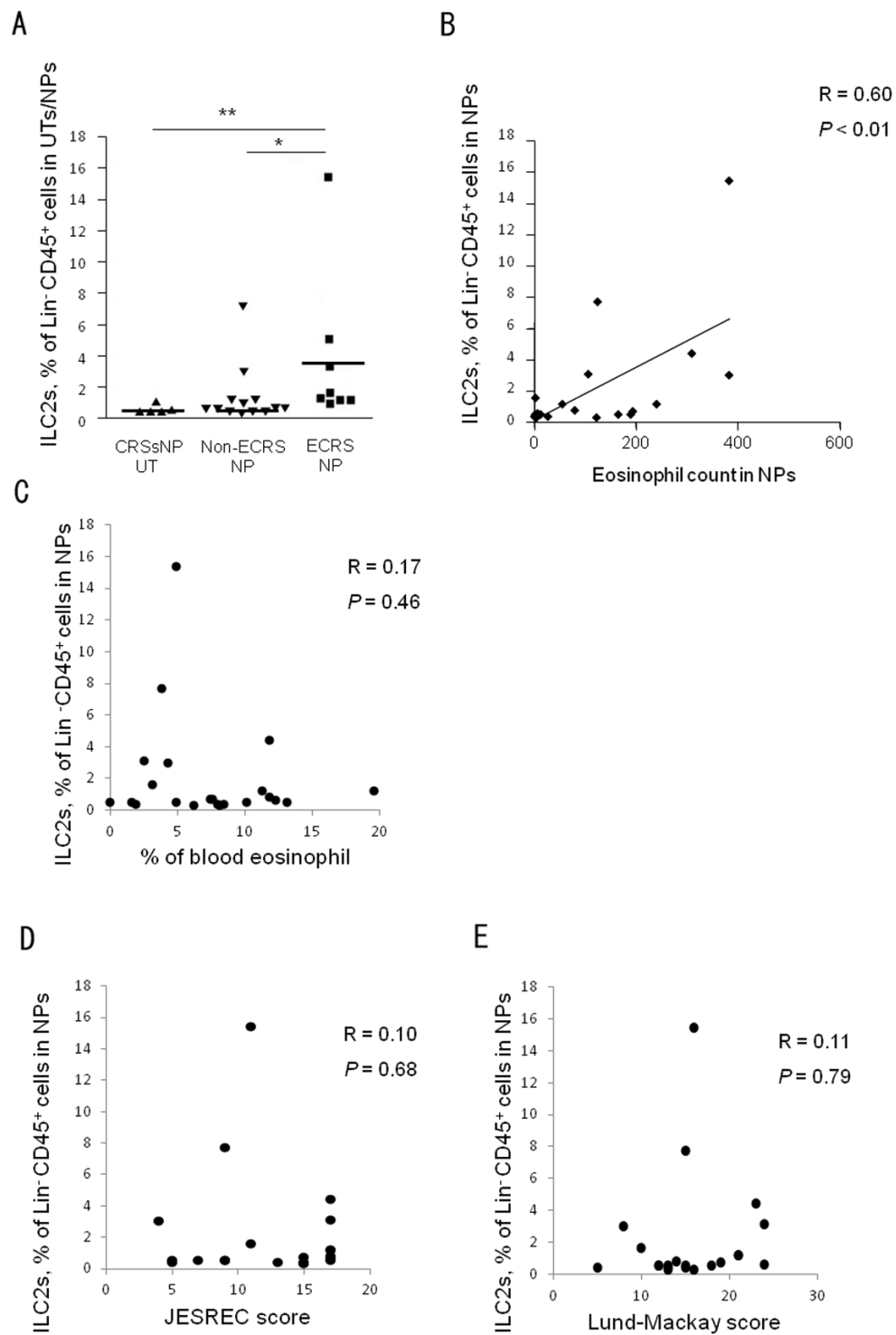


Figure 4

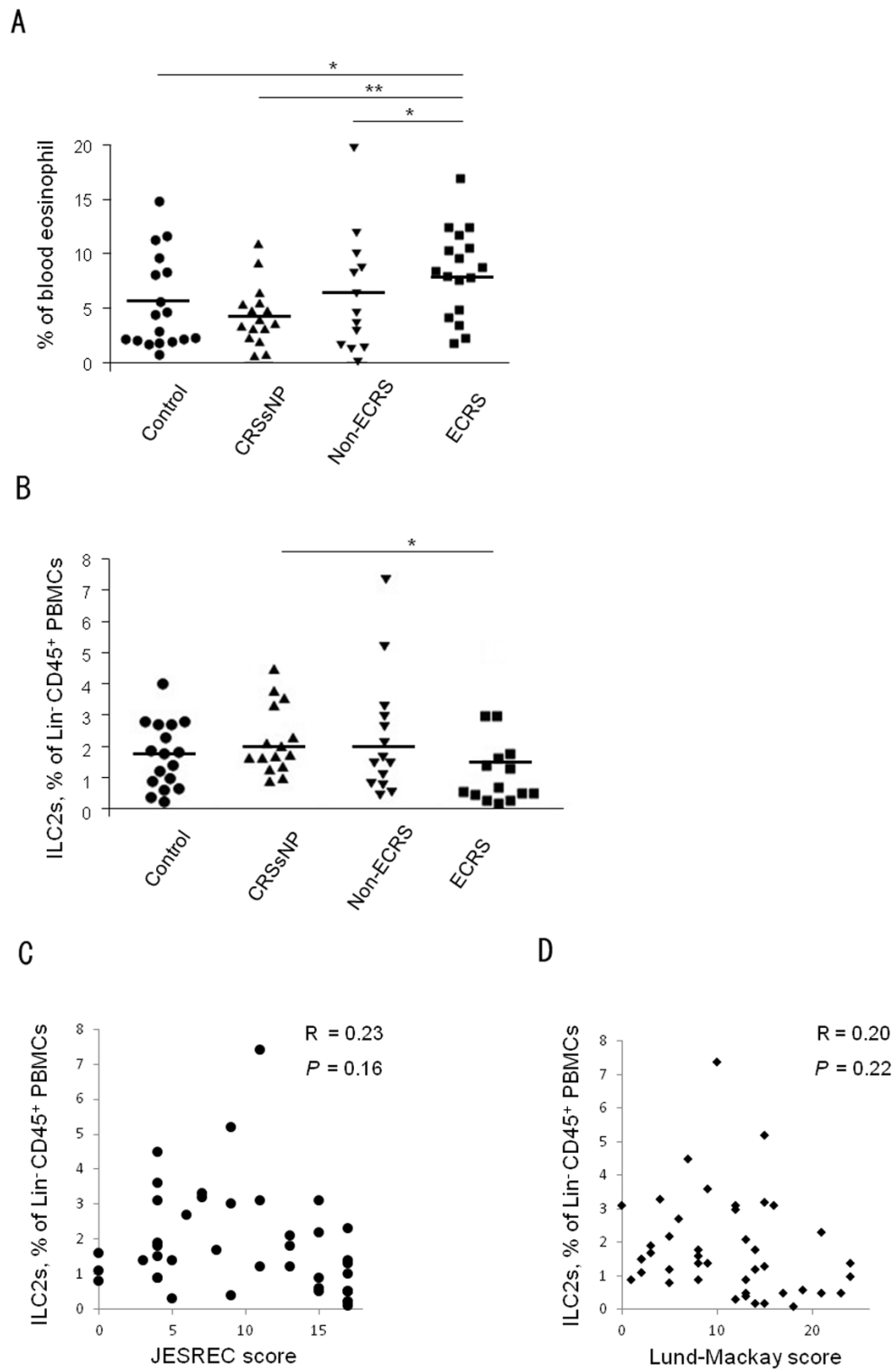


Figure 5

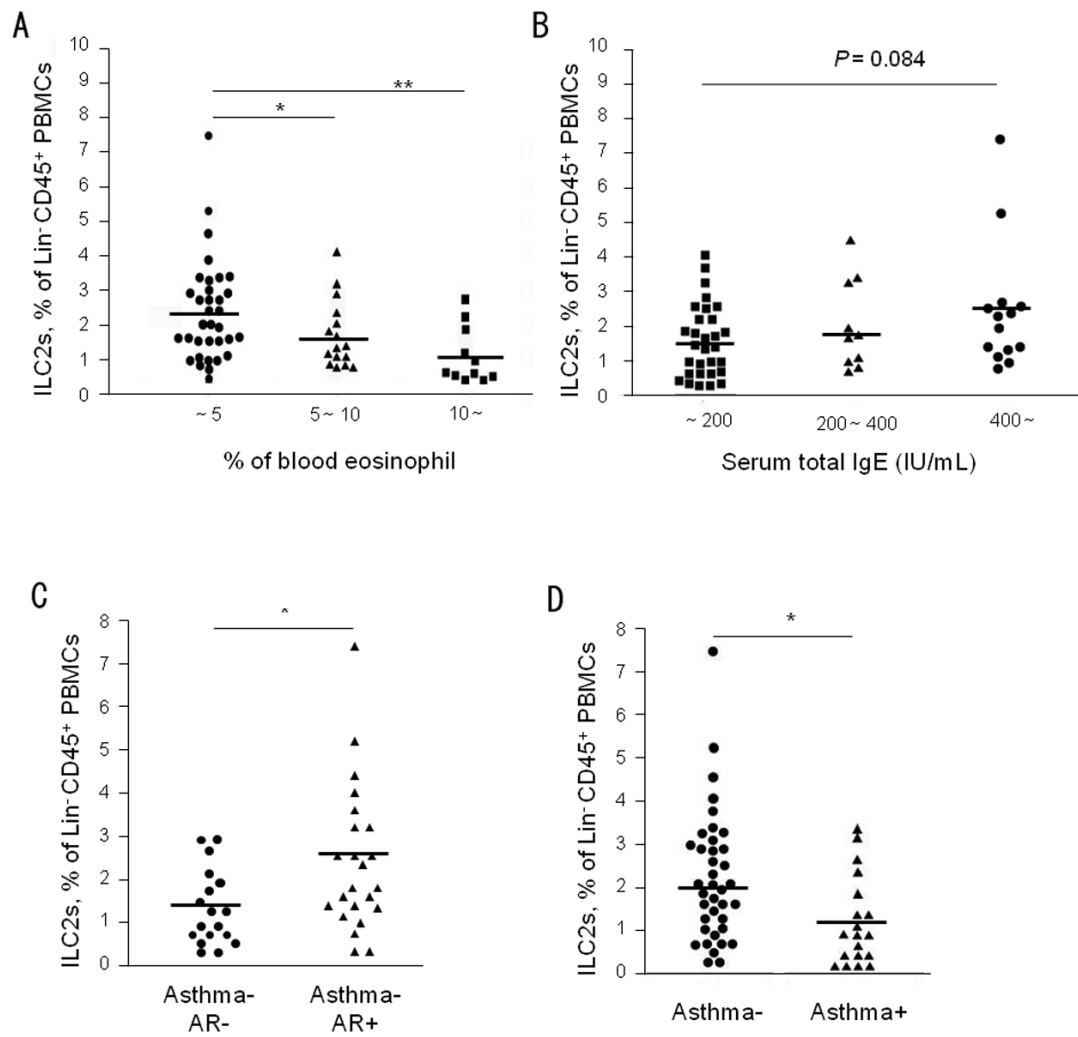


Figure 6

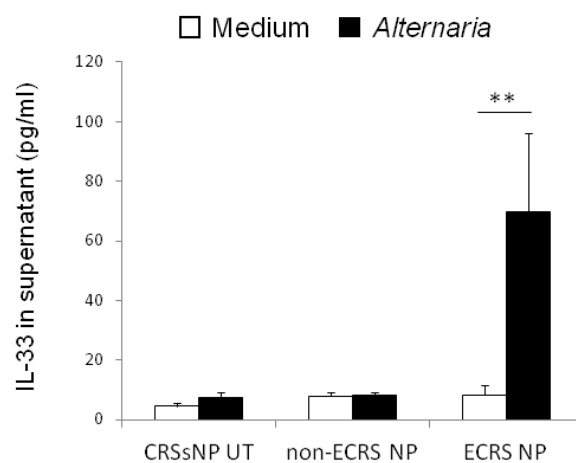


Table 1

	Control subjects	Patients with CRSsNP	Patients with CRSwNP	
			Non-ECRS	ECRS
	(n = 17)	(n = 16)	(n = 16)	(n = 14)
Age (y), mean $\pm$ SEM	36.8 $\pm$ 3.5	56.2 $\pm$ 4.4	50.9 $\pm$ 4.4	55.5 $\pm$ 3.5
Sex, male / female	10 / 7	10 / 6	10 / 6	7 / 7
Asthma (AIA), n	4 (0)	2 (0)	4 (0)	11 (2)
Allergic Rhinitis, n	11	11	9	7
% of blood eosinophil	5.3 $\pm$ 1.2	4.1 $\pm$ 0.7	5.7 $\pm$ 1.3	8.9 $\pm$ 0.8
Serum total IgE (IU/ml)	220 $\pm$ 60	206 $\pm$ 55	192 $\pm$ 55	217 $\pm$ 37
Lund-Mackay score	0	5.1 $\pm$ 0.9	12.6 $\pm$ 1.3	16.8 $\pm$ 1.0
JESREC score	0	5.0 $\pm$ 1.0	9.4 $\pm$ 1.2	15.6 $\pm$ 0.5

High lights:

- The prevalence of ILC2s in nasal polyps was high in patients with ECRS.
- The prevalence of ILC2s in blood was not different between ECRS and non-ECRS.
- The prevalence of ILC2s in blood was high in patients with allergic rhinitis.
- The prevalence of ILC2s in blood was low in patients with asthma.
- *Alternaria*-induced IL-33 secretion was increased in nasal epithelial cells of ECRS.