Innate immune crosstalk in asthmatic airways: Innate lymphoid cells coordinate polarization of lung macrophages

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Innate immune crosstalk in asthmatic airways:
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Background: Recent studies have emphasized the role of innate lymphoid cells (ILCs) in the development of asthma. The involvement of group 2 innate lymphoid cells (ILC2s) in asthma is well studied; however, the participation of other types of ILCs in the development of asthma remains unclear.

Objective: This study aims to understand the role of various ILCs in patients with asthma, especially their effect on macrophage polarization.

Methods: Each subset of ILCs and macrophages in induced sputum from 51 steroid-naive patients with asthma and 18 healthy donors was analyzed by using flow cytometry. Alveolar macrophages (AM) were sorted and cocultured with each subset of ILCs to determine whether the polarization of macrophages could be regulated by ILCs.

Results: In addition to ILC2s, numbers of group 1 innate lymphoid cells (ILC1s) and group 3 innate lymphoid cells (ILC3s) were increased in induced sputum from asthmatic patients when compared with those in healthy control subjects. The dominance of macrophages in induced sputum was more prominent in asthmatic patients than in healthy control subjects. A positive correlation between numbers of ILC2s and numbers of M2 macrophages and those of ILC1s/ILC3s and M1 macrophages was observed. Coculture of ILC2s with AMs induced expression of M2 macrophage–related genes, whereas coculture of ILC1s and ILC3s with AMs increased expression of M1 macrophage–related genes through cytokine secretion, as well as cell-cell contact. According to the inflammatory signature, patients with eosinophilic asthma have more ILC2s and M2 macrophages, and those with noneosinophilic asthma have an M1 macrophage–dominant profile.

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Conclusion: A different subset of ILCs regulates macrophage polarization, contributing to developing the distinct phenotype of asthma. (J Allergy Clin Immunol 2018;:——:—.)

Key words: Asthma, innate lymphoid cells, macrophage, polarization, induced sputum, asthma phenotype

Asthma is an immunologic disease of the airways characterized by airway hyperresponsiveness (AHR) and chronic airway inflammation related to an intricate network of diverse immune cells. Allergen-specific Th2 cells have long been thought to play critical roles in inducing type 2 cytokine production in the lungs, resulting in development of allergic asthma, the most common form of the disease. More recently, it has become clear that asthma is mediated by both innate and adaptive immune cells that regulate and shape pulmonary inflammation.

With increased awareness of asthma heterogeneity, researchers subdivide patients with asthma into 4 to 5 phenotypic clusters according to their age, sex, atopy, lung function, health care use, and body mass index. Among the cluster, patients with notable eosinophilia and a notable increase in levels of interleukins, such as IL-4, IL-5, and IL-13, in the blood are said to have eosinophilic asthma. Another group shows a minimal Th2 response but notable sputum neutrophilia with an increase in levels of IL-17A. Therefore understanding the mechanisms underlying each asthma phenotype will foster the development of improved and more appropriate therapeutic strategies for asthma.

Recently identified innate lymphoid cells (ILCs) have been found to link innate components of immunity to adaptive immunity. ILCs play critical roles in host defense and tissue homeostasis, particularly in mucosal tissues. Although ILCs lack antigen-specific T-cell and B-cell receptors, respectively, they rapidly produce a variety of cytokines in response to a wide range of innate signals: IL-25, IL-33, thymic stromal lymphopoietin, and IL-17A. Based on their characteristics and cytokine profiles, ILCs have been divided into 3 groups; group 1 innate lymphoid cells (ILC1s), group 2 innate lymphoid cells (ILC2s), and group 3 innate lymphoid cells (ILC3s), which can be thought of as the innate counterparts of CD4+ Th1, Th2, and Th17 cells. ILC1s produce IFN-γ and TNF-α. ILC2s produce a set of cytokines similar to those produced by Th2 cells, such as IL-5, IL-9, and IL-13. ILC3s produce IL-17A, IL-22, GM-CSF, and TNF-α. ILC3s display further heterogeneity and can be subdivided into several subgroups, such as CCR6+ lymphoid tissue inducers and CCR6− ILC3s. CCR6− ILC3s are further subdivided based on the expression of natural cytotoxicity receptor (NCR). RAR-related orphan receptor γt is a key transcription factor for ILC3 development; however, T-bet is also required for regulation of NKp46 expression on CCR6+ ILC3s. Interactions between ILCs and other immune cells are still under investigation; however, recent studies have shown that ILCs can demonstrate new mechanisms underlying immune responses, especially in mucosal tissues, such as the intestines and lungs.

Macrophages constitute the foremost controllers of both innate and acquired immunity. They are the most abundant immune cells in the lung (approximately 70% of total immune cells), and polarization of macrophages has been heavily associated with the development of allergic asthma. Macrophages can be polarized after exposure to microenvironmental stimuli and categorized as classically (M1) or alternatively (M2) activated phenotypes. Regulation of macrophage polarization involves a complex interplay between various cytokines, chemokines, and signaling molecules. IFN-γ and LPS are known to induce differentiation of M1 macrophages, whereas IL-4 and IL-13 upregulate expression of genes involved in M2 macrophage generation.

The importance of ILCs in the development of asthma is well defined in animal models of asthma and in patients with asthma. Conversely, interaction between ILCs and other innate immune cells has not been fully understood. This study aimed to identify the role of ILCs as mediators in the development of asthma by estimating their population in induced sputum from patients with asthma. In addition, correlation between numbers of ILCs and several innate immune cells, including macrophages, neutrophils, and eosinophils, was analyzed. Our data suggested that cytokine production from ILCs can coordinate the polarization of macrophages, which participate in the development of different types of asthma.

METHODS

Subjects

We recruited 51 asthmatic patients from the Department of Internal Medicine, Seoul National University Hospital, Seoul, South Korea, from September 2015 to September 2016. All patients had a history of repetitive respiratory symptoms, such as cough, dyspnea, or wheezing, and showed significant AHR on methacholine challenge (PC20 <16 mg/mL). Eighteen healthy donors (nonsmokers or ex-smokers of <10 pack-year) who were free of any respiratory symptoms and had normal lung function served as control subjects. All asthmatic patients were treatment naive. Sputum induction and sample processing were performed, as previously described. To distinguish patients with noneosinophilic from those with eosinophilic asthma, blood eosinophils were counted in whole blood samples from patients, and if eosinophil counts exceeded 300 cells/μL, patients were classified as having eosinophilic asthma.

All subjects enrolled in this study provided written informed consent, and the study protocol was approved by the Seoul National University Hospital Institutional Review Board (Institutional Review Board no. 1610-062-799).

Cell isolation from induced sputum

For eliminating mucus, we processed induced sputum with the same volume of 0.1% dithiothreitol (Sigma, St Louis, Mo) with shaking for

Abbreviations used

AHR: Airway hyperresponsiveness
AM: Alveolar macrophage
Arg1: Arginase 1
CD40L: CD40 ligand
CRTH2: Chemoattractant receptor–homologous molecule expressed on Tgfβ cells
ICOS: Inducible T-cell costimulator
ILC: Innate lymphoid cell
ILC1: Group 1 innate lymphoid cell
ILC2: Group 2 innate lymphoid cell
ILC3: Group 3 innate lymphoid cell
IL-7R: IL-7 receptor
Nos2: Nitric oxide synthase 2
Mrc1: Mannose receptor C type 1
NCR: Natural cytotoxicity receptor
PD-1: Programmed cell death protein 1
Retnla: Resistin-like molecule α
Flow cytometry

Single cells from sputum were blocked with anti-CD16/CD32 (BD Biosciences, Franklin Lake, NJ) and stained with fluorochrome-labeled mAbs directed against cell-surface markers for 1 hour at 4°C. For analysis of human induced sputum, the following antibodies were used. Anti-CD45 (HI30) was purchased from BD Biosciences. Anti-CD3e (UCHT1), anti-CD11c (3.9), anti-CD11b (ICRF44), anti-CD14 (HCD14), anti-CD15 (W6/32), anti-CD19 (HIB19), anti-CD49b (P1E6-C5), anti-CD68 (Y1/82A), anti-CD117 (104D2), anti-CD127 (A019D5), anti-CD25 (PC61) were from BioLegend. Flow cytometry was performed with the BD FACSAria.

Coculture of ILCs and macrophages

For expansion of ILC1s and ILC3s in vivo, 6- to 8-week-old mice were inoculated intratracheally with 100 ng of recombinant IL-33 (BioLegend). After 48 hours, supernatants were harvested for analysis of cytokine production by using ELISA, and AMs were harvested with TR1zol Reagent (Invitrogen, Carlsbad, Calif) for analysis of M1 and M2 gene expression by using quantitative RT-PCR.

Quantitative real-time PCR

Harvested AMs were homogenized with TR1zol Reagent. After homogenization, total RNA was extracted according to the manufacturer’s instructions. cDNA was synthesized with the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, Calif). RT-qPCR assays were performed with IQ Supermix (Bio-Rad Laboratories). Relative expression levels were calculated with normalizing quantification cycle values of target genes to quantification cycle values of glyceraldehyde-3-phosphate dehydrogenase (Gapdh). All primers were purchased from Thermo Fisher Scientific (TaqMan Gene Expression Assays; Thermo Fisher Scientific, Upssala, Mass).

ELISA

ELISA for supematant IFN-γ, IL-4, and IL-9 (BD Bioscience) and IL-5, IL-13, and IL-17 (R&D systems, Minneapolis, Minn) were performed, according to the manufacturer’s instructions.

Statistical analysis

Before comparison, normality tests were performed with Shapiro-Wilk normality tests. Comparisons of 2 groups were performed by using the Mann-Whitney test if the data were nonparametric or with the unpaired t test if the data were parametric. Multiple groups were compared by using the Kruskal-Wallits test, followed by a Dunn posttest if the data were nonparametric or 1-way ANOVA followed by a Bonferroni posttest.

RESULTS

Numbers of all types of ILCs, as well as ILC2s, were increased in induced sputum of asthmatic patients

The presence of ILCs in induced sputum was evaluated in 51 patients with asthma and 18 healthy control subjects (Table I). Total ILCs were initially gated as CD45+ Lineage- CD127+ ST2+ CD25+ lymphoid cells. ILC2s were defined as CD45+ Lineage- CD127+ ST-2+ CD25+ lymphoid cells. AMs were stained with anti-CD45, anti-CD49b, and anti-CD11c antibodies for 30 minutes. Cells were sorted with the BD FACSAria.

Coculture of ILCs and macrophages

Sorted AMs (2.5 × 10^5 cells) were cultured in the lower chamber of a 24-well transwell plate (0.4-μm pore; Corning, Tewksbury, Mass). After 24 hours, freshly sorted ILC1s and ILC3s or sorted ILC2s (5 × 10^4 cells) were added in the upper chamber of the transwell. In some experiments ILCs were added in the same chamber of macrophages.

Recombinant IL-2 (20 U/mL; BioLegend), IL-12 (20 ng/mL; BioLegend), and IL-23 (20 ng/mL; BioLegend) or IL-2 (20 U/mL) and IL-33 (20 ng/mL; BioLegend) were added for ILC1/ILC3 or ILC2 stimulation, respectively. In some experiments anti–programmed cell death protein 1 (PD-1; 10 μg/mL; BioXCell, West Lebanon, NH), anti–inducible T-cell costimulator (ICOS; 10 μg/mL; BioXCell), and anti–CD40 ligand (CD40L) blocking antibodies (10 μg/mL; BioXCell) were used to block cell-cell contact between AMs and ILCs. After 48 hours, supernatants were harvested for analysis of cytokine production by using ELISA, and AMs and ILCs were harvested with TR1zol Reagent (Invitrogen, Carlsbad, Calif) for analysis of M1 and M2 gene expression by using quantitative RT-PCR.
Increase in numbers of ILC2s and M2 macrophages in induced sputum of patients with eosinophilic asthma

The patients were divided into 2 groups based on the number of eosinophils to investigate potential associations between various populations of ILCs and macrophages and the phenotypes of asthma (see Table E1 in this article’s Online Repository at www.jacionline.org). ILC2 percentages were significantly increased in induced sputum of patients with eosinophilic asthma (P < .0001; Fig 2, A) when compared with those of patients with noneosinophilic asthma. AM percentages were similar in sputum from patients with eosinophilic asthma, whereas an increased number of M2 macrophages was observed in patients with noneosinophilic asthma (M1, P < .05; M2, P < .0001; AMs, P < .0001; Fig 2, C) when compared with the control subjects. Alterations in the number of macrophages were not associated with lung function (FEV1/forced vital capacity ratio), airway hypersensitivity (PC20 level), or numbers of WBCs (see Fig E4, A–C, in this article’s Online Repository at www.jacionline.org). Among the population of macrophages, AMs had a strong negative correlation with M1 (P = .0001; Fig 2, D) and M2 (P < .0001; Fig 2, E) macrophages, indicating that AMs might be differentiated into the M1 or M2 phenotypes based on the type of stimulus in asthmatic patients.

TABLE I. Characteristics of patients

<table>
<thead>
<tr>
<th>No. of patients (n)</th>
<th>Healthy control subjects</th>
<th>Asthmatic patients</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>51</td>
<td></td>
<td>.0899</td>
</tr>
<tr>
<td>Age (y)</td>
<td>52.93 ± 7.57</td>
<td>57.49 ± 14.30</td>
<td></td>
</tr>
<tr>
<td>Symptom duration (y)</td>
<td>ND</td>
<td>4.14 ± 5.36</td>
<td></td>
</tr>
<tr>
<td>FEV1 (L)</td>
<td>2803 ± 607.4</td>
<td>2096 ± 665.7</td>
<td>.0006</td>
</tr>
<tr>
<td>FEV1 (%)</td>
<td>103.5 ± 12.73</td>
<td>87.69 ± 14.04</td>
<td>.0003</td>
</tr>
<tr>
<td>FVC (%)</td>
<td>3516 ± 785.5</td>
<td>2853 ± 891.6</td>
<td>.0085</td>
</tr>
<tr>
<td>FVC (%)</td>
<td>107.3 ± 13.09</td>
<td>95.61 ± 15.61</td>
<td>.0129</td>
</tr>
<tr>
<td>FEV1/FVC ratio</td>
<td>0.8 ± 0.06</td>
<td>0.73 ± 0.08</td>
<td>.0040</td>
</tr>
<tr>
<td>PC20 (mg/mL)</td>
<td>ND</td>
<td>4.73 ± 3.74</td>
<td>—</td>
</tr>
<tr>
<td>Atopy, no. (%)</td>
<td>0 (0)</td>
<td>18 (35.3)</td>
<td></td>
</tr>
<tr>
<td>Allergic rhinitis, no. (%)</td>
<td>0 (0)</td>
<td>28 (54.9)</td>
<td></td>
</tr>
</tbody>
</table>

Induced sputum differential count

<table>
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<th></th>
<th>Healthy control subjects</th>
<th>Asthmatic patients</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophages (%)</td>
<td>42.07 ± 26.03</td>
<td>57.67 ± 14.09</td>
<td>.0059</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>39.93 ± 28.39</td>
<td>22.59 ± 13.60</td>
<td>.0508</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0.9 ± 1.57</td>
<td>15.22 ± 13.39</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC (×10³/L)</td>
<td>ND</td>
<td>6834 ± 1777</td>
<td>—</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>ND</td>
<td>5.45 ± 3.96</td>
<td>—</td>
</tr>
<tr>
<td>Total IgE (IU/mL)</td>
<td>ND</td>
<td>238.39 ± 323.7</td>
<td>—</td>
</tr>
</tbody>
</table>

Data are presented as means ± SDs.

FVC, Forced vital capacity; ND, no data.

healthy control subjects (P < .001; see Fig E1, A, in this article’s Online Repository at www.jacionline.org). Percentages and absolute numbers of total ILCs were increased in induced sputum from patients with asthma compared with those in healthy donors (P < .01; Fig 1, A and B, and see Fig E1, B). In addition to the ILC2s, numbers of both ILC1s and NCR−ILC3s were also significantly increased in the asthmatic patients (P < .0001; Fig 1, C). However, the increase in numbers of ILCs was not related to the pulmonary function index (FEV1/forced vital capacity), airway hypersensitivity (PC20 level), and WBC count (see Fig E2, A–C, in this article’s Online Repository at www.jacionline.org). Negative correlations were observed between ILC1 and NCR−ILC3 counts (P < .0001; Fig 1, D) and NCR+ILC3 counts (P < .05; Fig 1, E), which might be due to the transition between ILC1s and ILC3s reported in human subjects. However, we could not see any correlations between other subsets of ILCs (see Fig E2, D). Thus far, only ILC2s have been known to be increased in asthmatic patients; however, our data clearly showed that all types of ILCs were increased in the induced sputum of asthmatic patients.

Numbers of CD68+ macrophages were increased in induced sputum from asthmatic patients

We hypothesized that an increase in the number of ILCs might result in crosstalk between them and other immune cells, especially innate immune cells, such as AMs. First, we checked the population and composition of macrophages in induced sputum from asthmatic patients and analyzed a subset of macrophages using flow cytometry. CD45+CD68+ cells were first gated as macrophages and then categorized as M1 and M2 macrophages based on the expression of CD11c and CD206, respectively (Fig 2, A). The M1 and M2 macrophages presented with different expression levels of HLA-DR (see Fig E3, B and C, in this article’s Online Repository at www.jacionline.org). Macrophages that expressed both CD11c and CD206 were considered AMs.33 Percentages and absolute numbers of total macrophages were increased in induced sputum of asthmatic patients when compared with those in healthy control subjects (P < 0.0001; Fig 2, B, and see Fig E3, A); moreover, each subgroup of macrophages was increased in the induced sputum of asthmatic patients (M1, P < .05; M2, P < .0001; AMs, P < .0001; Fig 2, C) when compared with the control subjects. Alterations in the number of macrophages were not associated with lung function (FEV1/forced vital capacity ratio), airway hypersensitivity (PC20 level), or numbers of WBCs (see Fig E4, A–C, in this article’s Online Repository at www.jacionline.org).
patients with eosinophilic asthma and not in patients with noneosinophilic asthma (*P < .001; Fig 3, C).

Respiratory diseases are known to influence asthma outcome and phenotype.\textsuperscript{34-36} Patients were classified into 3 different groups based on the presence or absence of allergic rhinitis and chronic rhinosinusitis to assess whether the comorbidity of upper airway disease in asthmatic patients affects the distribution of ILCs and other immune cells (see Table E2 in this article’s Online Repository at www.jacionline.org). No differences between the subtypes of ILCs and macrophages (see Fig E5 in this article’s Online Repository at www.jacionline.org) were noted. However, the number of NCR\textsuperscript{−}ILC3s was decreased in sputum from asthmatic patients with allergic rhinitis and chronic rhinosinusitis (*P < .01; see Fig E5, B).

**Distribution of innate immune cells in sputum is different and related to asthma phenotype**

To verify the presence of associations between asthma phenotypes and the distribution of innate immune cells, we...
FIG 2. Macrophage counts are increased in induced sputum of asthmatic patients. A, Representative macrophage gating strategy from induced sputum of healthy donors (n = 18) and asthmatic patients (n = 51). Total macrophages (CD68+ cells), M1 macrophages (CD68+ CD11c+ CD206- cells), M2 macrophages (CD68+ CD11c- CD206- cells), and AMs (CD68+ CD11c- CD206+ cells). B, Comparison of total macrophage frequencies (percentage of CD45+ cells) between healthy donors and asthmatic patients. C, Comparison of M1 macrophage, M2 macrophage, and AM frequencies (percentage of CD45+ cells) in induced sputum of healthy donors and asthmatic patients. D, Correlation between M1 macrophage and AM frequencies (percentage of macrophages) in induced sputum of asthmatic patients. E, Correlation between M2 macrophage and AM frequencies (percentage of macrophages) in induced sputum of asthmatic patients. Mann–Whitney or Student t tests were used to compare healthy control subjects and asthmatic patients, and the Pearson correlation test was used to measure associations between variables. Values represent means ± SDs. *P < .05 and ****P < .0001.
compared the relationships of innate immune cells in induced sputum of asthmatic patients. Interestingly, patients with greater numbers of eosinophils in sputum presented with increased numbers of ILC2s (P < .05; Fig 4, A). Unlike ILC2s, there were no correlations between numbers of ILC1s, NCR\textsuperscript{+} ILC3s, or NCR\textsuperscript{-} ILC3s and eosinophils (Fig 4, A) and numbers of other subsets of ILCs and neutrophils in induced sputum (see Fig E6, A, in this article’s Online Repository at www.jacionline.org).

Next, we examined the relationship between macrophages and other innate immune cells. Patients with increased counts of eosinophils in induced sputum demonstrated an increase in M2 macrophage percentages (P < .01), whereas M1 macrophage

FIG 3. Patients with eosinophilic asthma have type 2 airway inflammation. A, Frequencies of each subset of ILCs (percentage of CD45\textsuperscript{+} cells) in induced sputum of patients with noneosinophilic and eosinophilic asthma. B, M1 macrophage, M2 macrophage, and AM frequencies (percentage of CD45\textsuperscript{+} cells) in induced sputum of patients with noneosinophilic and eosinophilic asthma. C, Gene expression of IFN-\textgamma, IL-5, IL-13, and IL-17A in pellets of whole sputum cells. *P < .05, **P < .01, and ***P < .001, Mann-Whitney test to compare between the patients with noneosinophilic and eosinophilic asthma. Values represent means \pm SDs.
percentage did not show correlation with eosinophil counts (see Fig E6, B). Conversely, neutrophil counts in sputum were positively correlated with M1 macrophage counts \((P < .05)\) and negatively correlated with M2 macrophage counts \((P < .001; \text{see Fig E6, C})\).

We further investigated the presence of associations between ILC and macrophage counts because alterations in these 2 populations are most significant in asthmatic patients. Interestingly, ILC1 and ILC3 percentages were positively correlated with increases in percentages of M1 macrophages \((P < .01 \text{ and } P < .05, \text{respectively; Fig 4, B})\) but not M2 macrophages. On the other hand, asthmatic patients who had more ILC2s presented with more M2 macrophages in induced sputum \((P < .01; \text{Fig 4, C})\). However, in healthy donors no such correlations were observed (data not shown). These data suggest that ILCs and macrophages interact with each other in a cell-specific manner, and some of these interactions could alter the outcome of asthma.

**ILCs can promote macrophage polarization by secreting cytokines and through direct interactions**

On the basis of current results, we hypothesized that specific types of ILCs affect macrophage polarization. To test this hypothesis, we cocultured sorted ILCs with AMs (see Fig E7, A–C, in this article’s Online Repository at www.jacionline.org). ILC2s were stimulated with recombinant IL-2 and IL-33, whereas ILC1s and ILC3s were stimulated with recombinant IL-2, IL-12, and IL-23 (Fig 5, A). Coculture of AMs with ILC1s and ILC3s significantly induced mRNA expressions of M1 macrophage–related genes, such as nitric oxide synthase 2 \((\text{Nos2})\), \(\text{Cd86}\), and prostaglandin-endoperoxide synthase 2 \((\text{Ptgs2})\); \(P < .001, P < .0001, \text{and } P < .001, \text{respectively; Fig 5, B})\). In contrast, mRNA expression of M2 macrophage–related genes, such as arginase 1 \((\text{Arg1})\) and resistin-like molecule \(\alpha\) \((\text{Retnla})\), were increased when AMs were cocultured with ILC2s \((P < .0001 \text{ and } P < .001, \text{respectively; Fig 5, C})\). It is well known that macrophage
FIG 5. ILCs affect AM polarization in vitro. A, Schematic diagram of the coculture procedure. B, Expression of the M1-related genes Nos2, Cdx86, prostaglandin-endoperoxide synthase 2 (Ptgs2) in AMs cocultured with ILCs. C, Expression of the M2-related genes Arg1, Retnla, and Mrcl in AMs cocultured with ILCs. Gene expression data were normalized with glyceraldehyde-3-phosphate dehydrogenase (Gapdh) expression. D, IFN-γ and IL-17 expression levels in the ILC1/ILC3 and AM–cocultured group. E, Expression levels of the type 2 cytokines IL-4 and IL-13 in the ILC2 and AM–cocultured group. *P < .05, ***P < .001, and ****P < .0001, Kruskal–Wallis test, followed by the Dunn multiple comparison test. Data are representative of 3 independent experiments analyzed and presented as means ± SEMs.
FIG 6. ILC-macrophage interaction affects polarization of macrophages through cell-cell contact. A, Schematic diagram of the coculture procedure using the transwell system. B and C, Expression of M1-related (Nos2, Cd86, and prostaglandin-endoperoxide synthase 2 [Ptgs2]; Fig 6, B) and M2-related (Arg1, Retnla, and Mrc1; Fig 6, C) genes in AMs cocultured with ILC1s/ILC3s with and without the transwell insert. D and E, Expression of M1-related (Fig 6, D) and M2-related (Fig 6, E) genes in AMs cocultured with ILC2s with and without the transwell insert. Each gene expression was normalized to glyceraldehyde-3-phosphate dehydrogenase (Gapdh) expression. F, IFN-γ and IL-17 production in the ILC1/ILC3 and AM–cocultured group. G, IL-4 and IL-13 production in the ILC2 and AM–cocultured group. * P < .05, ** P < .01, *** P < .001, and **** P < .0001, Mann–Whitney test. Data are representative of 3 independent experiments and analyzed and presented as means ± SEMs.
polarization is regulated by cytokines. Therefore we presumed that ILCs might be involved in this process by secreting cytokines.

To examine cytokine production by ILCs, we measured IFN-γ, IL-17A, IL-4, and IL-13 levels in co-culture supernatants (Fig 5, D and E). Treatment with recombinant IL-12 and IL-23 stimulated ILC1s and ILC3s, thereby increasing levels of IFN-γ (P < .0001) and IL-17A (P < .05; Fig 5, D). Furthermore, stimulation of ILC2s with recombinant IL-33 resulted in increased levels of IL-4 and IL-13 (P < .001 and P < .001, respectively; Fig 5, E). In addition to IL-4 and IL-13, ILC2s produced IL-5 and IL-9 with recombinant IL-33 stimulation (P < .0001 and P < .5, respectively; see Fig E8 in this article’s Online Repository at www.jacionline.org). These results clearly show that cytokines from different subsets of ILCs can induce macrophage polarization.

Next, the transwell system was used to investigate whether soluble cytokines from ILCs affected macrophage polarization. As shown in Fig 6, A, AMs were cultured in the lower chamber, and different subsets of ILCs were then added to the upper chamber. After 48 hours, AMs from the lower chamber were harvested, and expression levels of M1 and M2 macrophage–related genes were analyzed. Unexpectedly, mRNA expression levels of M1 macrophage–related genes (Nos2 and Cd86) were decreased (P < .001 and P < .05, respectively; Fig 6, B) when AMs were cocultured with ILC1s and ILC3s in the transwell. Moreover, the mRNA expression level of one of the M2 macrophage–related gene, Arg1 was reduced and that of mannose receptor C type 1 (Mrc1) was increased (P < .0001 and P < .01; Fig 6, E) when AMs were cocultured with ILC2s in the transwell. However, expression levels of M1- and M2-related genes were not affected by coculture with ILC2s and ILC1s/ILC3s, respectively (Fig 6, C and D). IFN-γ and IL-17A levels in culture supernatants from the transwell were significantly lower than those without the transwell (IFN-γ, P < .05; IL-17A, P < .05; Fig 6, F). However, IL-4, IL-5, IL-9, and IL-13 levels remained unchanged in the presence of the transwell (Fig 6, G, and see Fig E8, B).

To investigate which surface molecules mediate the interaction of ILCs and macrophages, we treated cells with anti–PD-1, anti-ICOS, and anti-CD40L, which are costimulatory or inhibitory molecules expressed by ILCs. When we cocultured AMs and ILC1/ILC3s with blocking antibodies, there were no changes on M1 and M2 macrophage–related gene expression (see Fig E9, A and B, in this article’s Online Repository at www.jacionline.org). Although blocking antibody treatment seems to reduce Arg1 and Retnla expression in the AM and ILC2 coculture group (Arg1, not significant; Retnla, P < .0001; see Fig E9, C and D), we did not find surface molecules that can regulate macrophage polarization.

Taken together, these data suggest that specific types of ILCs regulate the polarization of AMs by cytokine secretion, as well as cell–cell contact. The opposite might also hold true because cytokine secretion from ILCs was decreased when ILCs were separated from AMs. Further studies clarifying the precise mechanisms involved are merited.

DISCUSSION

This study has demonstrated the distribution of innate immune cells (ILCs) within the airways of asthmatic patients, along with several additional novel findings. First, in addition to ILC2s, other types of ILCs were also increased in the sputum of asthmatic patients. Second, ILC2 and M2 macrophage counts were increased in patients with eosinophilic asthma, whereas ILC1, ILC3, and M1 macrophage counts were increased in patients with noneosinophilic asthma. Third, specific types of ILCs could induce macrophage polarization differently, thereby affecting the pathogenesis of asthma.

The discovery of ILCs in the lungs has provided a breakthrough in our understanding of cell types and innate pathways underlying asthma development. ILCs are an emerging type of immune cells that are Lineage− but produce large amounts of effector cytokines. Furthermore, the presence of ILC1s and ILC3s in the sputum of asthmatic patients is correlated with disease severity. Thus far, only ILC2s have been implicated in the development of allergic asthma; however, other types of ILCs also appear to play important roles in different forms of asthma. In support of this hypothesis, the current study showed that numbers of all types of ILCs were significantly increased in the sputum of patients with asthma when compared with those in healthy control subjects. This diverse distribution of ILCs may reflect the heterogeneous nature of asthma.

To the best of our knowledge, this is the first study to report the presence of ILC1s and ILC3s in the sputum of asthmatic patients, unlike previous studies that focused on ILC2s only. Although the function of ILCs in the development of asthma was originally identified in mice, several human studies have demonstrated their roles in the development of this condition. Bartemes et al reported that the prevalence of ILC2s was increased in PBMCs from patients with allergic asthma when compared with healthy control subjects. Nagakumar et al demonstrated the presence of ILC2s in the airways of children with severe therapy-resistant asthma. They first identified ILC2s in induced sputum, where the proportion was found to be greater than that in peripheral blood.

Although several studies have been reported regarding the role of ILCs in asthmatic patients, there are several limitations. First, most studies examined ILCs by using PBMCs rather than bronchoalveolar lavage fluid or induced sputum. As shown by Gasteiger et al, PBMCs do not reflect actual lung environments where ILCs are known to reside. In the current study we demonstrated the presence of ILCs in induced sputum, which might be a more accurate reflection of the profile of ILCs rather than that in blood.

Second, there are a limited number of immune cells in the sputum. However, we tried to collect adequate amounts of sputum from the patients to analyze the accurate population of the immune cells and were able to confirm the interactions between macrophages and ILCs in the present study.
Third, previously, all experiments were focused only on ILC2s, and no other cell types were identified; however, in the current study all types of ILCs were examined and found to be increased in the induced sputum of asthmatic patients. Moreover, the ILCs were associated with macrophage polarization in sputum. Although numbers of all types of ILCs were increased in the sputum from asthmatic patients, a negative correlation between ILC1 counts and NCR^+ ILC3 or NCR^− ILC3 counts was observed. It is well known that NCR^− ILC3s and ILC1s exhibit plasticity in the intestine.31 It is also reported that ILC2s and ILC1s demonstrate plasticity in the lungs of mice with chronic obstructive pulmonary disease.56,57; nonetheless, the conversion of ILC1s and ILC3s in the lung was not clear. Because ILC1 and ILC3 plasticity was not observed in healthy control subjects in the current study (data not shown), certain inflammatory conditions in asthmatic patients might be responsible for induction of ILC plasticity.

The proportion of ILC2s in sputum from patients with eosinophilic asthma was greater than that from patients with noneosinophilic asthma. A positive correlation between ILC2 and blood eosinophil counts was observed, whereas no such positive correlations were observed between ILC1 and ILC3 and neutrophil counts. Alternatively, the M1 macrophage counts in sputum were positively correlated with neutrophil counts, whereas M2 macrophage proportions were positively correlated with eosinophil counts. These findings suggest that ILCs might directly affect the polarization of macrophages, which could regulate infiltration of eosinophils or neutrophils into the airways.

To confirm this hypothesis, we analyzed other immune cells from sputum and found that an increase in the number of macrophages was correlated with the number of ILCs. In other words ILC2s had a positive correlation with M2 macrophages, whereas both ILC1s and ILC3s demonstrated positive correlations with M1 macrophages. Coculture experiments using mouse ILCs and AMs showed that activated ILCs could regulate the polarization of macrophages by secreting cytokines, as well as cell–cell contact. M1 macrophages can be differentiated by IFN-γ, whereas the key cytokines that activate M2 macrophages are IL-4 and IL-13.22,58 Therefore IL-4 and IL-13 from ILC2s could induce polarization of M2 macrophages, whereas IFN-γ or IL-17A from ILC1s and ILC3s could induce polarization of M1 macrophages. Among markers for M2 macrophages, gene expression of Mrc1 was downregulated in AMs cocultured with ILC2s (Fig 5, C). Mrc1 is one of the typical markers for M2 macrophages, which is upregulated by IL-4 and IL-13 stimulation.59 However, in tissue-resident macrophages expression of Mrc1 is independent of IL-4 and IL-13.59 AMs are lung-resident macrophages, and therefore IL-4 and IL-13 released from ILC2s might not affect Mrc1 expression. Also, because AMs highly expressed Mrc1 at steady state (Fig 2, A), we know that Mrc1 expression in AMs was regulated independent of IL-4 and IL-13. In addition, M2 macrophages do not constitute a uniform population and are further subdivided into M2a, M2b, and M2c macrophages. MRC1 is expressed in M2a and M2c but not in M2b macrophages.60 Therefore decrease in MRC1 might indicate a change in the M2 subtype although further analysis need to be done.

The roles of M1 and M2 macrophages in the development of asthma differ from each other. M1 macrophages are the major effector cells in patients with nonallergic forms of asthma, whereas M2 macrophages are predominant in patients with the allergic forms of asthma.61 Although the current M1/M2 paradigm with its polarized extremes is oversimplified, our data showed that certain subsets of ILCs could regulate the polarization of macrophages. More interestingly, macrophage polarization was only induced when macrophages were cocultured with ILCs. The combination of recombinant cytokines did not affect macrophage polarization, indicating the importance of ILCs during this process. Thus the ILC-macrophage axis might be important to induce the development of different types of asthma.

It is worthy of note that polarized M1/M2 macrophages could induce ILC activation. M1 macrophages induce T_h1 cell activation through TNF-α and IL-12 production and by interacting with CD86 and MHC class II molecules, thereby affecting the development of nonallergic asthma.62,63 Similarly, M2 macrophages activate T_h2 cells through IL-4 and IL-13 production and by interacting with CCL17 and MRC1 and lead to the development of allergic asthma.64 There is a possibility that ILCs and macrophages interact in a cell–contact manner, and the inhibition of this interaction in the transwell system might have reduced cytokine production by ILCs. Recently, it has been reported that ILCs also expresses costimulatory or inhibitory molecules, such as PD-1, ICOS, and CD40L, on their surfaces.65-67 Activated AMs also express high levels of programmed death ligand 1 and CD40.66,67 Therefore we hypothesized that ILCs and macrophages could interact through these molecules. When we blocked the ILC1/ILC3-macrophage interaction using anti-PD-1, anti-ICOS, and anti-CD40L antibodies, M1 and M2 macrophage–related genes were not changed (see Fig E9, A and B). That means unknown molecules, not PD-1, ICOS, and CD40L, might mediate cell-cell interaction between ILC1/3s and AMs. In case of ILC2-macrophage interaction, Arg1 and Retnla expression was downregulated by these blocking antibodies (Fig E9, C and D). However, gene expression of Retnla tended to be increased when we cocultured ILC2s and macrophages separately using the transwell system. This discrepancy between the experiments (ie, coculture without transwell or with transwell) might indicate that other unknown molecules could mediate interaction of ILC2s and AMs. Although we could not show the surface molecules that mediate the interaction between ILCs and macrophages in current study, we believe that identifying these interactions will be important for understanding the features of ILCs and macrophages.

Although we have found a close relationship between ILCs and macrophages according to asthma phenotype, we did not see the correlation between ILCs and macrophages with AHR (see Fig E2). As far as we know, there was no report about the correlation between ILCs and AHR in human asthmatic patients. One reason why we could not see the positive relationship might be because of the sample we obtained. In our study we recruited newly diagnosed and treatment-naıve patients to exclude the interference effects of drugs on immune cells. Therefore most patients we recruited belong to patients with mild asthma, with FEV1 percent predicted at greater than 80%. Because AHR was caused by severe inflammation and airway damage, it is hard to see the correlation between AHR and immune cells in our samples, which are mostly from patients with mild asthma. Also, it is possible that ILCs and macrophages are not direct mediators of AHR. The inflammatory factors known to induce AHR are histamine and leukotrienes, and these proteins are mostly released from eosinophils and mast cells.65 We found a negative correlation between AHR and eosinophil counts (data not shown). Perhaps increased ILCs...
or macrophages could regulate AHR through interaction with other immune cells, such as eosinophils and mast cells.

In conclusion, for the first time, this study shows that in addition to ILC2s, various other ILCs are also increased in the airways of asthmatic patients. These ILCs might be essential sources of cytokines that induce macrophage polarization and control the development of airway eosinophilia or noneosinophilic inflammation. Understanding the factors that regulate the activation of ILCs might be important for the treatment of patients with difficult-to-control asthma because ILCs can crosstalk with several innate immune cells. ILCs might play key roles in controlling allergic or nonallergic inflammation in asthmatic patients during the early stages of asthma development. However, more experiments evaluating the relationships between ILCs, macrophages, and other immune cells in the lungs are warranted.

Key messages
- Asthmatic patients have more significant number of ILCs in induced sputum than healthy donors.
- ILC2–M2 macrophage numbers are increased in patients with eosinophilic asthma, whereas ILC1/ILC3–M1 macrophage counts are increased in patients with noneosinophilic asthma.
- ILC2s induce M2 macrophage polarization, and ILC1s/ILC3s induce the M1 macrophage polarization through cytokine secretion, as well as cell–cell contact.

REFERENCES
47. Agache I, Ciobanu C, Agache C, Anghel M. Increased serum IL-17 is an independent risk factor for severe asthma. Respir Med 2010;104:1131-7.
FIG E1. Frequency of CD45⁺ cells and absolute numbers of ILCs in induced sputum. A, Percentage of total CD45⁺ cells in induced sputum. B, Absolute number of total ILCs in induced sputum. **P < .01 and ***P < .001, Mann–Whitney test. Values represent means ± SDs.
FIG E2. Correlation between clinical data and percentages of each group of ILCs in induced sputum. Percentages of ILCs based on flow cytometric analysis, as described in Fig 1. A, Correlation between FEV₁/forced vital capacity (FVC) ratio and ILCs. B, Correlation between PC20 values and ILCs. PC20 values represent the concentration of methacholine chloride leading to a 20% decrease in FEV₁ level. C, Correlation between WBC count per microliter and ILCs analyzed. WBC counts were based on complete blood cell count results. D, Correlation between numbers of ILC2s and other ILCs or between NCR⁻ILC3s and NCR⁻ILC3s. n.s, Not significant (Pearson correlation test).
FIG E3. Comparison of expression levels of HLA-DR in M1 and M2 macrophages. A, Absolute numbers of total macrophages in induced sputum. B, Mean fluorescence intensity (MFI) levels of HLA-DR in M1 and M2 macrophages. C, Histogram of HLA-DR expression levels in M1 and M2 macrophages. ****P < .0001, Mann–Whitney test. Values represent means ± SDs.
FIG E4. Correlation between clinical data and percentages of macrophages in induced sputum. Percentages of macrophages were calculated on the basis of flow cytometric analysis, as described in Fig 2. A, Correlation between FEV\textsubscript{1}/FVC ratio and macrophage numbers. B, Correlation between PC\textsubscript{20} values and macrophage numbers. C, Correlation between WBC counts per microliter and macrophage numbers. n.s., Not significant (Pearson correlation test).
FIG E5. Asthmatic patients with upper airway diseases have lower NCR^1 ILC3s in induced sputum. Comparison among asthmatic patients based on the presence of allergic rhinitis (AR) and chronic rhinosinusitis (CRS) is shown. A, Frequencies of total ILCs (percentage of CD45^1 cells) in induced sputum. B, Frequencies of each subset of ILCs (percentage of CD45^1 cells) in induced sputum. C, Frequencies of total numbers of macrophages (percentage of CD45^1 cells) in induced sputum. D, Frequencies of M1 macrophages, M2 macrophages, and AMs (percentage of CD45^1 cells) in induced sputum. **P < .01, Kruskal–Wallis test, followed by the Dunn multiple comparison test. Values represent means ± SDs.
FIG E6. Correlations between ILC and neutrophil numbers and correlations between macrophage and eosinophil or neutrophil numbers in induced sputum. A, Correlation between numbers of each subset of ILCs and neutrophils. B, Correlation between eosinophil numbers and numbers of each subset of macrophages in induced sputum analyzed. C, Correlation between neutrophil numbers and numbers of each subset of macrophages in induced sputum analyzed. *P < .05 and ***P < .001, Pearson correlation test. n.s., Not significant.
FIG E7. Sorting strategy of ILCs and macrophages. Purity of the cells after fluorescence-activated cell sorting with the BD FACS Aria were as follows: 

A, non-ILC2s (CD45^+ Lineage^+ CD127^+ ST-2^+ CD25^+ ), 97.3%; 

B, ILC2s (CD45^+ Lineage^+ CD127^- ST-2^- CD25^- ), 96.2%; and 

C, AMs (CD45^- F4/80^- CD11c^- ), 97.7%.
FIG E8. Levels of IL-5 and IL-9 in coculture of ILC2s and AMs. A, Protein levels of IL-5 and IL-9 in supernatants of coculture of ILC2s and AMs. B, Comparison of protein levels of IL-5 and IL-9 in the ILC2 and AM coculture system with and without transwell. *P < .05, **P < .01, ***P < .001, and ****P < .0001, Mann–Whitney test or 1-way ANOVA, followed by the Bonferroni posttest. Data are representative of 2 independent experiments and presented as means ± SEMs.
FIG E9. Changes in gene expression related to M1 and M2 macrophages in AMs cocultured with ILCs by using anti–PD-1, anti-ICOS, and anti-CD40L blocking antibodies. A, Gene expression of M1-related genes in AMs cocultured with ILC1s/ILC3s by using anti–PD-1, anti-ICOS, and anti-CD40L. B, Gene expression of M2-related genes in AMs cocultured with ILC1s/ILC3s by using anti–PD-1, anti-ICOS, and anti-CD40L. C, Gene expression of M1-related genes in AMs cocultured with ILC2s by using anti–PD-1, anti-ICOS, and anti-CD40L. D, Gene expression of M2-related genes in AMs cocultured with ILC2s by using anti–PD-1, anti-ICOS, and anti-CD40L. **P < .01, ***P < .001, and ****P < .0001, 1-way ANOVA followed by a Bonferroni posttest. Data are representative of 2 independent experiments and presented as means ± SEMs.
TABLE E1. Comparison of characteristics between patients with noneosinophilic and those with eosinophilic asthma

<table>
<thead>
<tr>
<th></th>
<th>Noneosinophilic asthma</th>
<th>Eosinophilic asthma</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients (n)</td>
<td>21</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>60.86 $\pm$ 13.53</td>
<td>54.76 $\pm$ 14.29</td>
<td>.0496</td>
</tr>
<tr>
<td>Symptom duration (y)</td>
<td>4.105 $\pm$ 3.79</td>
<td>3.186 $\pm$ 3.79</td>
<td>.2940</td>
</tr>
<tr>
<td>FEV$_1$</td>
<td>91.38 $\pm$ 15.57</td>
<td>85.10 $\pm$ 13.37</td>
<td>.1682</td>
</tr>
<tr>
<td>FVC</td>
<td>2669 $\pm$ 722.8</td>
<td>2973 $\pm$ 1096</td>
<td>.2954</td>
</tr>
<tr>
<td>FVC (%)</td>
<td>96.52 $\pm$ 20.01</td>
<td>94.24 $\pm$ 11.20</td>
<td>.6503</td>
</tr>
<tr>
<td>FEV$_1$/FVC ratio</td>
<td>0.7369 $\pm$ 0.07</td>
<td>0.7448 $\pm$ 0.08</td>
<td>.7403</td>
</tr>
<tr>
<td>PC$_{20}$ (mg/mL)</td>
<td>6.080 $\pm$ 4.49</td>
<td>3.630 $\pm$ 3.01</td>
<td>.0552</td>
</tr>
<tr>
<td>Smoking history (never/ex-smoker/current), no. (%)</td>
<td>17 (81.0)/2 (9.5)/2 (9.5)</td>
<td>16 (76.2)/5 (23.8)/0 (0)</td>
<td></td>
</tr>
<tr>
<td>Macrophages (%)</td>
<td>55.81 $\pm$ 12.67</td>
<td>59.61 $\pm$ 14.22</td>
<td>.3786</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>31.07 $\pm$ 13.49</td>
<td>15.06 $\pm$ 7.98</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>6.123 $\pm$ 6.58</td>
<td>23.48 $\pm$ 12.96</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Induced sputum differential count</td>
<td>17 (81.0)/2 (9.5)/2 (9.5)</td>
<td>16 (76.2)/5 (23.8)/0 (0)</td>
<td></td>
</tr>
<tr>
<td>WBC ($\times 10^9$/L)</td>
<td>6575 $\pm$ 1587</td>
<td>7093 $\pm$ 1954</td>
<td>.3512</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>2.490 $\pm$ 1.36</td>
<td>8.414 $\pm$ 3.44</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Total IgE (IU/mL)</td>
<td>187.5 $\pm$ 321.7</td>
<td>295.8 $\pm$ 341.2</td>
<td>.1977</td>
</tr>
</tbody>
</table>

Data are presented as means $\pm$ SDs.

FVC, Forced vital capacity.
TABLE E2. Comparison of characteristics between asthmatic patients and asthmatic patients with other respiratory diseases

<table>
<thead>
<tr>
<th></th>
<th>Asthma</th>
<th>Asthma + AR</th>
<th>Asthma + AR + CRS</th>
<th>P value</th>
</tr>
</thead>
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<tr>
<td>No. of patients (n)</td>
<td>15</td>
<td>16</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>60.67 ± 10.15</td>
<td>53.38 ± 20.21</td>
<td>56.75 ± 8.38</td>
<td>.3813</td>
</tr>
<tr>
<td>Symptom duration (y)</td>
<td>4.027 ± 3.61</td>
<td>3.988 ± 4.63</td>
<td>6.633 ± 8.32</td>
<td>.5891</td>
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<tr>
<td>FEV$_1$</td>
<td>1985 ± 514.2</td>
<td>2133 ± 807.7</td>
<td>2093 ± 641.7</td>
<td>.8201</td>
</tr>
<tr>
<td>FEV$_1$ (%)</td>
<td>88.73 ± 14.13</td>
<td>90.81 ± 14.90</td>
<td>84.00 ± 14.21</td>
<td>.4656</td>
</tr>
<tr>
<td>FVC</td>
<td>2806 ± 781.1</td>
<td>2821 ± 990.7</td>
<td>2799 ± 935.8</td>
<td>.8828</td>
</tr>
<tr>
<td>FVC (%)</td>
<td>93.33 ± 17.99</td>
<td>99.63 ± 15.59</td>
<td>94.00 ± 12.01</td>
<td>.4808</td>
</tr>
<tr>
<td>FEV$_1$/FVC ratio</td>
<td>0.7102 ± 0.08</td>
<td>0.7525 ± 0.09</td>
<td>0.7518 ± 0.05</td>
<td>.2432</td>
</tr>
<tr>
<td>PC$_{20}$ (mg/mL)</td>
<td>4.767 ± 4.53</td>
<td>4.944 ± 3.86</td>
<td>3.508 ± 2.22</td>
<td>.7167</td>
</tr>
<tr>
<td>Smoking history (never/ex-smoker/current), no. (%)</td>
<td>11 (73.3%/4 (26.7)/0 (0)</td>
<td>14 (87.5%/0 (0)/2 (12.5)</td>
<td>10 (83.3%/2 (16.7)/0 (0)</td>
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<td>Induced sputum differential count</td>
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<td></td>
</tr>
<tr>
<td>Macrophages (%)</td>
<td>55.35 ± 15.12</td>
<td>59.81 ± 15.92</td>
<td>57.06 ± 13.35</td>
<td>.6521</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>21.87 ± 10.82</td>
<td>22.63 ± 13.17</td>
<td>17.58 ± 11.44</td>
<td>.3606</td>
</tr>
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<td>Peripheral blood</td>
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</tr>
<tr>
<td>WBC ($\times 10^3$/L)</td>
<td>6775 ± 1654</td>
<td>7272 ± 2022</td>
<td>6413 ± 1435</td>
<td>.5047</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>4.175 ± 3.48</td>
<td>6.227 ± 4.38</td>
<td>6.811 ± 4.46</td>
<td>.1920</td>
</tr>
<tr>
<td>Total IgE (IU/mL)</td>
<td>86.13 ± 85.02</td>
<td>228.3 ± 300.4</td>
<td>418.8 ± 482.9</td>
<td>.1020</td>
</tr>
</tbody>
</table>

Data are presented as means ± SDs.

AR, Allergic rhinitis; CRS, Chronic rhinosinusitis; FVC, forced vital capacity.