

IL-13⁺ Type 2 Innate Lymphoid Cells Correlate with Asthma Control Status and Treatment Response

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Abstract

Type 2 innate lymphoid cells (ILC2s) have been shown to produce large amounts of type 2 cytokines in a non-antigen-specific manner. These cytokines act upstream and downstream of ILC2 and are increasingly common in asthma drug development, thus warranting a closer investigation of the mechanism-related clinical manifestations of ILC2 in the selection of patients with asthma. We hypothesized that IL-13⁺ ILC2s in the circulation might correlate with asthma control status as a result of persistent T-helper cell type 2 (Th2) inflammation in the lung. Furthermore, we aimed to explore ILC2s' responsiveness to glucocorticoid. The percentages of ILC2s and IL-13⁺ ILC2s in different asthma subgroups were checked, and correlation analyses between ILC2s and asthma-related clinical parameters were performed. Dexamethasone treatments in ILC2s and Th2 cells were performed to clarify their response properties. ILC2s were identified as a Lin⁻CD45^{hi}IL-7Rα⁺CRTH2⁺ cell population distinct from human peripheral blood mononuclear cells. Frequencies of ILC2s were increased dramatically in those with asthma (0.04 ± 0.02%) compared with healthy donors (0.025 ± 0.011%). The percentages of IL-13⁺ ILC2s were significantly higher in patients in the uncontrolled group (49.7 ± 16.9%) and partly controlled groups (30.8 ± 13.1%) than in those in the well-controlled group (16.7 ± 5.9%) and healthy control subjects (18.7 ± 8.7%). Effective treatment of uncontrolled IL-13⁺ ILC2-positive patients with asthma

resulted in dynamic modulation of IL-13⁺ ILC2 levels back to baseline. ILC2s were more resistant to glucocorticoid than Th2 cells *in vitro*. ILC2s are strong responders to IL-25/IL-33 stimulation. IL-13⁺ ILC2s show a positive correlation with patient asthma control status and are more resistant to glucocorticoid than Th2 cells in humans.

Keywords: ILC2; innate immunity; IL-13; uncontrolled asthma; glucocorticoid

Clinical Relevance

We demonstrate that IL-13⁺ type 2 innate lymphoid cells (ILC2s) in peripheral blood associate with asthma control status but not with other asthma-related clinical parameters. Successful treatment of patients with asthma with uncontrolled IL-13⁺ ILC2s results in dynamic modulation of IL-13⁺ ILC2 levels to baseline. We also confirm that peripheral ILC2s were more resistant to glucocorticoid than CD4⁺ T-helper cells type 2 in humans. This study may influence drug development relating to upstream/downstream components of ILC2s and have potential value in glucocorticoid-resistant asthma.

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Asthma is a common chronic inflammatory disease of the airway. Currently, >300 million people suffer from asthma worldwide, and at least 250,000 deaths attributed to the disease each year (1–4). Asthma is characterized by strong heterogeneity, with differences in environments and individuals significantly affecting the development and exacerbation of the disease (5, 6). In 2014, the Global Initiative for Asthma (GINA) provided a global strategy for asthma management and prevention, which was updated in 2015 (1, 2). These new guidelines were used to assess asthma control status according to patients' clinical symptoms in the preceding 4 weeks, together with the risk of future exacerbations, and the development of fixed airflow limitations or medication side effects. On the basis of these guidelines, asthma can be separated into uncontrolled, partly controlled, and well-controlled subgroups.

Innate lymphoid cells (ILCs) are groups of non-T, non-B lymphoid-like cells. These cells can elicit prompt responses to external stimulation in the absence of antigen-specific receptors on their surface. On the basis of the types of cytokines produced, ILCs can be divided into three major types (ILC1 [7, 8], ILC2 [7, 9], and ILC3 [10, 11]) that are similar to T-helper cell type 1 (Th1), Th2, and Th17 cells, respectively. In them, ILC2s exert innate immune function by producing large amounts of type 2 cytokines, especially IL-5, IL-9, and IL-13, in response to IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) stimulation (12–17). These cells have been implicated in antiinflammatory and antiinfectious processes to protect against extracellular parasites such as helminths. Recent studies indicate that mouse ILC2s can also promote the expression of amphiregulin to participate in lung tissue repair (17), regulate eosinophil homeostasis (18, 19), drive the development of alternatively activated macrophages (20), induce airway hyperresponsiveness (AHR) (21), and play critical roles in viral- or allergy-induced lung inflammation and asthma (22, 23). Kabata and colleagues further demonstrated that TSLP played a pivotal role in the steroid-resistant induction of ILC2s by controlling STAT5 phosphorylation and Bcl-xL expression in the mouse (24).

Together with the exciting findings in mouse ILC2s, their contribution to human diseases has emerged gradually. Mjösberg and colleagues first reported that human

ILC2s could be induced by IL-25 and IL-33 in the fetal gut and identified CRTH2 and CD161 as important surface markers (14). Salimi and colleagues further discovered that the numbers of ILC2s were increased dramatically in the localized areas of patients with atopic dermatitis and that RNA levels of ST2, ROR α , and amphiregulin were enhanced. Moreover, IL-33 could stimulate skin ILC2s to produce IL-4, IL-5, and IL-13 (15). Bartemes and colleagues found that the prevalence of human ILC2s in peripheral blood was greater in those with allergic asthma than in patients with allergic rhinitis and in healthy control patients (25). Christianson and colleagues found the percentage of ILC2s and IL-13⁺ILC2s in bronchoalveolar lavage fluids from patients with asthma was significantly higher than in control subjects, and that these cells were essential for asthmatic persistence (26). Furthermore, Smith and colleagues demonstrated that the number of activated ILC2s was increased significantly in the airways of patients with severe asthma, and that these cells could also promote the persistence of airway eosinophilia (27).

ILC2's potential role as an early source of type 2 cytokines and its involvement in persistent asthma make it an attractive cell type for therapeutic intervention. Furthermore, patient selection strategies that enable targeted application of these differentiated therapies are urgently needed. In this study, we sought to explore the mechanism-related clinical manifestations of ILC2 in human asthma, with the goal of broadening our knowledge of ILC2's roles in asthma pathogenesis, and thus aimed to provide new insight for future personalized treatments.

Materials and Methods

Participants

Peripheral blood samples from patients with asthma and from healthy donors were collected at Zhongshan Hospital at Fudan University. Some of the blood samples were obtained from Baogang Hospital. The eligibility and exclusion criteria are shown in the online supplement.

Asthma control status and severity were assessed using the 2014 GINA guidelines (see Table E1 in the online supplement). Disease history, eosinophil count, IgE, fractional exhaled nitric oxide

(F_{ENO}), pulmonary function test data, body mass index, medication, and acute exacerbation (AE) information were collected from each patient. Healthy subjects comprised adults between 18 and 75 years old with no history of allergic diseases or respiratory diseases. The protocol (No: B2014-108) was approved previously by the institutional review board at Fudan University. All subjects provided written informed consent.

Isolation and Processing of Peripheral Blood Mononuclear Cells

Peripheral blood mononuclear cells (PBMCs) were isolated using histopaque 1077 (Sigma-Aldrich, St Louis, MO) following the manufacturer's instructions. Plasma was collected and stored at –80°C for subsequent cytokine detection. PBMCs were washed twice with Dulbecco's phosphate-buffered saline and were used for fluorescence-activated cell sorter (FACS) staining.

Surface and Intracellular Staining of Human ILC2s

Isolated PBMCs were stained with FACS antibodies. Details of the ILC2 surface and intracellular staining methodologies are described in the online supplement.

Sorting and Functional Determination of ILC2s

ILC2s (Lin[–]CD45^{hi}IL-7R α ⁺CRTH2⁺ cells), Lin[–]CD45^{hi}IL-7R α ⁺CRTH2[–] cells, Lin[–]CD45^{hi}IL-7R α [–]CRTH2[–] cells, and Lin⁺CD45^{hi}IL-7R α ⁺CRTH2⁺ cells (staining protocol was the same as the one described in the online supplement) were sorted from PBMCs using a FACS Aria II machine, seeded into 384-well plates at a concentration of 5×10^4 /ml, and then cultured in complete RPMI 1640 in the presence of IL-2 (20 ng/ml) (R&D, Minneapolis, MN) or IL-2 + IL-25 (10 ng/ml) (R&D) + IL-33 (10 ng/ml) (R&D) for 6 days. Cell morphology was observed under a microscope, and supernatant was harvested and subjected to cytokine multiplex panel analysis (IL-4, IL-5, IL-6, IL-13, IL-17, IFN- γ , and TNF- α), as recommended by the manufacturer (EMD Millipore, Billerica, MA).

Correlation Analysis between ILC2s and Asthma Clinical Parameters

The percentage of IL-13⁺ILC2s was calculated from different subgroups of

patients with asthma. GINA scores, IgE levels, FEV₁ of % predicted, peripheral eosinophil counts, and F_{ENO} levels were also tested. Correlations between IL-13⁺ILC2 percentage and clinical parameters were analyzed (Prism 6, La Jolla, CA). *R* values and *P* values were calculated. Significance criteria are described below.

Effects of Dexamethasone on Peripheral Activated ILC2s and CD4⁺ Th2 Cells

The culture conditions used to assess the dexamethasone (DEX) response of activated ILC2s and CD4⁺ Th2 cells are described in the online supplement.

Statistical Analysis

All data were representative of at least three independent experiments. Results are expressed as mean $\pm$ SEM. A Student's two-tailed *t* test was used to determine the level of difference between the two groups. *P* values <0.05 were considered significant.

Results

Peripheral ILC2s Have the Potential to Produce Large Amounts of Type 2 Cytokines in Response to IL-25 + IL-33 Stimulation *In Vitro*

Because ILC2s represent a rare cell population within both peripheral and local sites, multiple surface markers were used to identify this rare cell type. On the basis of publications from Mjösberg and colleagues (14), Salimi and colleagues (15), and Bartemes and colleagues (25), the lineage markers, CD45, IL-7R α , and CRTH2, were chosen to enable accurate ILC2 identification. Using these markers, one distinct cell population was identified as Lin⁻CD45^{hi}IL-7R α ⁺CRTH2⁺ cells from the PBMCs of healthy donors (Figure 1A).

To further qualify this population as ILC2s, we performed functional assays by cell sorting and compared them with other cell populations. The gating strategy for this approach is shown in Figure 1B. One thousand five hundred cells from each group were stimulated for 6 days using the key mediators, IL-25 and IL-33. Only Lin⁻CD45^{hi}IL-7R α ⁺CRTH2⁺ cells were found to have undergone significant morphological change after 6 days of culture. These cells became more

aggregated and enlarged in comparison to the cells in other groups (Figure 1C). Furthermore, cytokine-producing levels were evaluated using a multiplex ELISA panel. The data showed that only Lin⁻CD45^{hi}IL-7R α ⁺CRTH2⁺ cells could produce significant amounts of IL-5 (1,594 pg/ml) and IL-13 (3,457 pg/ml) and detectable levels of IL-4 (68 pg/ml) and IL-6 (38 pg/ml). IFN- γ and IL-17 levels were below our detection limit (Figure 1D), further confirming that the Lin⁻CD45^{hi}IL-7R α ⁺CRTH2⁺ cells were ILC2s and that these cells had the capacity to produce high levels of type 2 cytokines.

Percentages of Peripheral ILC2s Are Significantly Increased in Patients with Asthma Compared with Healthy Donors

Patients with asthma and healthy donors were recruited from the Respiratory Division of Zhongshan Hospital at Fudan University. Detailed patient information and clinical parameters in three subgroups and healthy donors are shown in Table 1. No significant differences were found in sex and age characteristics between the asthma subgroups and healthy subjects. All subjects had normal body weights, with a body mass index that varied from 23.0 to 24.9. A total of 53.8 to 69.2% of patients with asthma had allergic diseases such as allergic rhinitis, allergic conjunctivitis, or eczema, or had a positive skin prick test; this was a significant increase compared with that of healthy volunteers (*P* < 0.05), but there was no difference among asthma subgroups. The AE rate was gradually enhanced according to the severity of control status; the patients with asthma in the uncontrolled group showed a significantly higher AE rate (38.4%) than did the patients in the well-controlled group (11.5%) (*P* < 0.05). No statistical difference was found in other clinical parameters in the asthma subgroups, including FEV₁% predicted, F_{ENO}, eosinophil count, and IgE level.

The percentages of peripheral ILC2s were tested using multicolor FACS staining. A total of 0.04 $\pm$ 0.02% of ILC2s were detected in the lymphocytes from patients with asthma, which was dramatically higher than the number from healthy donors (0.025 $\pm$ 0.011%) (Figure 2A). However, no significant differences were found when comparing the ILC2 percentages in asthma subgroups (Figure 2B).

Percentages of IL-13⁺ILC2s, but not IL-5⁺ILC2s, Are Enhanced Dramatically in the Peripheral Blood of Patients with Uncontrolled/Partly Controlled Asthma Compared with That of Those with Well-Controlled Asthma and Healthy Donors

ILC2s are known to perform many functions in triggering immune responses, both systematically and locally (16). The secretion of type 2 cytokines, especially IL-5 and IL-13, represents one of their important functions (25). Accordingly, we investigated the intracellular IL-5 and IL-13 expression levels in ILC2s and overlaid this with ILC2 frequency data from the different asthma subgroups. Representative data for peripheral IL-13⁺ILC2 levels in patients with asthma and healthy donors are shown in Figure 3A. IL-13 expression was stained successfully from ILC2s and, interestingly, we found that the percentage of IL-13⁺ILC2s was decreased in accordance with the reduction of asthma severity in control status (Figure 3B). Totals of 44.7 $\pm$ 17.7% and 31.6 $\pm$ 12.2% of IL-13⁺ILC2s were identified in patients with uncontrolled and partly controlled asthma, respectively. In contrast, IL-13⁺ILC2 numbers were 16.7 $\pm$ 5.9% and 17.8 $\pm$ 8.6% in those with well-controlled asthma and healthy donors, respectively. These differences were significant. A similar analysis was performed to evaluate the IL-5⁺ILC2s' percentage in each group; however, no significant change was observed in samples from any of the study groups (see Figure E1). Therefore, IL-13⁺ILC2s were used for all subsequent experiments.

Frequency of IL-13⁺ILC2s Correlates Positively with Asthma Control Status

To confirm our hypothesis, we monitored the patients with asthma in the uncontrolled group for an extended time period. After treatment with long-acting β 2-agonist plus inhaled corticosteroid, theophylline, Chinese herbs, leukotriene receptor antagonist, oral glucocorticoid, or antihistamine drug for 3 to 4 months, the patients were recruited again for follow-up testing. The results showed that five patients were transitioned back to the well-controlled stage on the basis of the evaluation of their clinical symptoms. In addition, IL-13⁺ILC2s' frequencies in these five subjects were all decreased coincidentally with the amelioration of asthma control status (Figure 3B).

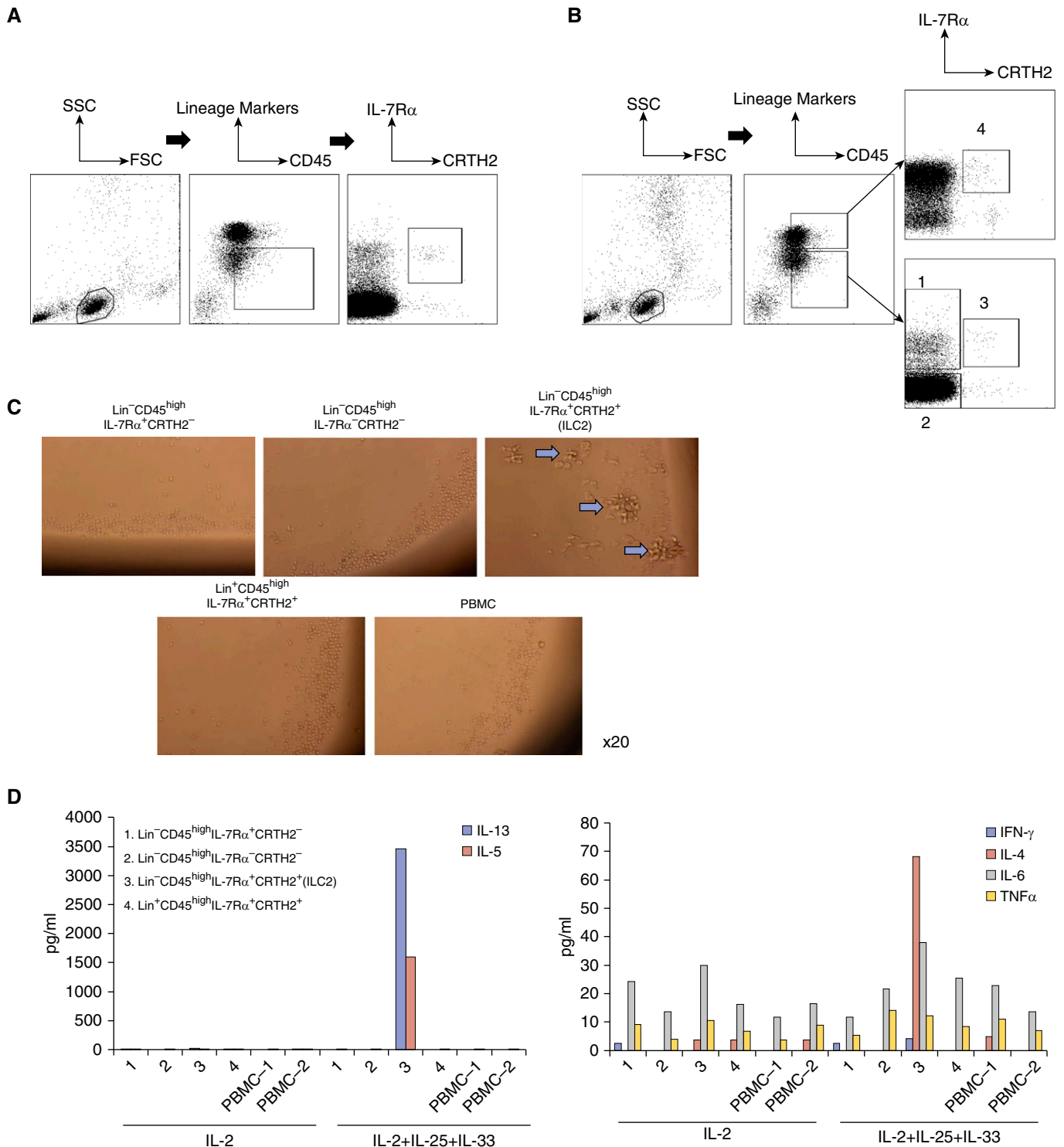


Figure 1. Identification and functional study of human type 2 innate lymphoid cells (ILC2s) in response to IL-25⁺IL-33 stimulation *in vitro*. (A) Identification of ILC2s from human peripheral blood using multi-fluorescence-activated cell sorter staining. (B) Sorting strategy of ILC2s from human peripheral blood. (C) Morphology changes of ILC2s after 6 days of stimulation with IL-25⁺IL-33. Arrows indicate activated ILC2 cells that become flat and form clusters. (D) Cytokine-release profile of ILC2s after 6 days of stimulation with IL-25⁺IL-33. Data are representative of results from at least three independent experiments. PBMC, peripheral blood mononuclear cell. CRTH2, chemoattractant receptor-homologous molecule expressed on Th2 cells; FSC, forward scatter; SSC, side scatter.

Table 1. Clinical Characteristics of Patients with Asthma and Healthy Control Subjects in The Study

Characteristics	Patients with Uncontrolled Asthma (n = 13)	Patients with Partly Controlled Asthma (n = 13)	Patients with Well-Controlled Asthma (n = 26)	Healthy Control Subjects (n = 34)
Sex, male (female)	7 (6)	9 (4)	12 (14)	16 (18)
Age, yr	51.5 ± 13.0	50.5 ± 15.6	52.0 ± 13.9	48 ± 12.5
History, yr	11.5 ± 16.4	6.3 ± 8.9	16.3 ± 15.9	ND
Allergy, %	7 (53.8)*	9 (69.2)*	17 (65.4)*	4 (11.7)
BMI, kg/m ²	24.9 ± 2.1	24.7 ± 1.9	23.0 ± 1.3	23.1 ± 1.8
FEV ₁ , %	72.0 ± 27.0	71.4 ± 25.4	74.2 ± 23.8	ND
F _{ENO} , ppb	46.6 ± 25.7	42.4 ± 27.6	38.9 ± 24.9	ND
Eosinophils, ×10 ⁹ /L	0.30 ± 0.16	0.35 ± 0.22	0.29 ± 0.21	ND
IgE, IU/ml	293 ± 261	642 ± 1473	273 ± 404	ND
Acute exacerbation, %	5 (38.4) [†]	3 (23.1)	3 (11.5)	ND

Definition of abbreviations: BMI, body mass index; F_{ENO}, fractional exhaled nitric oxide; ND, not determined.

Data are presented as mean ± SEM or n (%).

*P < 0.05 compared with healthy donors.

[†]P < 0.05 compared with patients with well-controlled asthma.

We performed further analyses between IL-13⁺ILC2 levels and clinical parameters of patients with asthma, including GINA score, IgE levels, eosinophil counts, FEV₁ predicted, F_{ENO}, and asthma history. As shown in Figure 4A, the levels of IL-13⁺ILC2s highly correlated with GINA score ($R = 0.79$, $P < 0.01$). No strong correlations were identified with other clinical parameters (Figures 4B–4F).

Activated ILC2s Are More Resistant to DEX Than Are CD4⁺ Th2 Cells

Having identified that IL-13⁺ILC2s associated positively with asthma control status, we deemed it necessary to do additional mechanistic studies to interpret

this phenomenon. In a mouse model, Kabata and colleagues showed that ILC2s are more resistant to corticosteroids than are Th2 cells (24). Because glucocorticoid resistance is a common phenomenon in patients with uncontrolled asthma (28), we sought to explore whether patient-derived ILC2s also exhibited steroid resistance.

Three doses of DEX (20, 100, and 500 nM), together with IL-2, IL-25, and IL-33 were used to treat 1×10^6 /ml of PBMCs from healthy donors for 5 days. The ability of DEX to inhibit ILC2 function was assessed by monitoring cell percentage changes in IL-13⁺ILC2s. The results showed that IL-13⁺ILC2 percentages in the 20-, 100-, and 500-nM DEX groups were

$40 \pm 9.9\%$, $35.4 \pm 9.8\%$, and $27.9 \pm 3.8\%$, respectively, compared with $32.4 \pm 5.1\%$ in the nontreatment group. Although a slight decrease was seen in the IL-13⁺ILC2 percentage with increasing DEX dosage, no significant difference was found in any group (Figure 5).

In parallel, CD4⁺ T cells were isolated from human PBMCs and differentiated into Th2 cells. The effect of DEX on Th2 cells was evaluated by the percentage of IL-13⁺Th2 cells. The percentages of IL-13⁺Th2 cells were $6.9 \pm 2.5\%$, $5.8 \pm 2.2\%$, and $5.9 \pm 1.7\%$ in the 20-, 100-, and 500-nM DEX-treated groups, respectively, compared with $12.9 \pm 4.8\%$ in the non-DEX treated group. Inhibition of Th2-cell function was gauged by the cell percentage change in IL-13⁺Th2 cells normalized by the value in the non-DEX treatment group. The results were $45.2 \pm 10.3\%$, $53.8 \pm 13.6\%$, and $51.3 \pm 12.4\%$ in the 20-, 100-, and 500-nM DEX-treated groups, respectively, all of which represented significant decreases compared with the non-DEX group. Moreover, the inhibitory effect of DEX on Th2 cells was dramatically higher than the effect on ILC2s at each treatment dose (Figure 5). In conclusion, ILC2s were more resistant to DEX than were Th2 cells in humans. The representative data for the functional changes occurring in ILC2s and Th2 cells are shown in Figures E2 and E3.

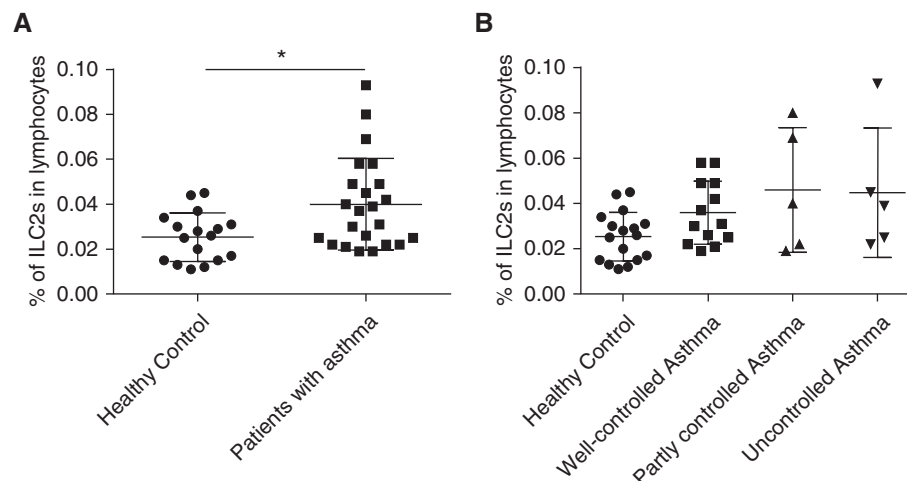


Figure 2. Percentage of ILC2s in the peripheral blood of patients with asthma. (A) ILC2s' percentage composition of lymphocytes in patients with asthma compared with healthy control subjects. (B) ILC2s' percentage composition of lymphocytes in patients with asthma with different control status compared with healthy control subjects. *P < 0.05 compared with healthy control subjects.

Discussion

Asthma is characterized pathophysiologically by airway inflammation, AHR, and

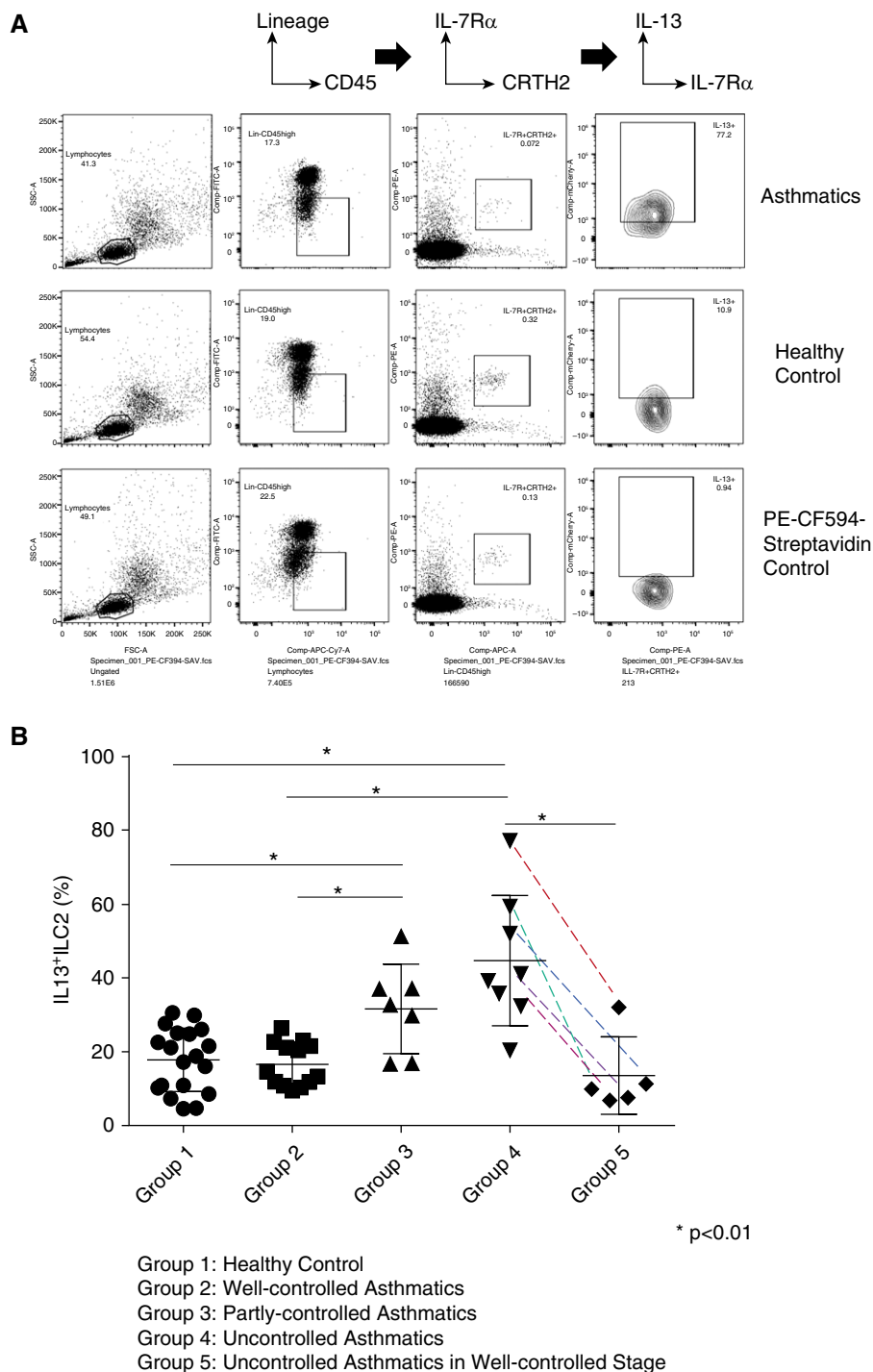


Figure 3. Percentage of IL-13⁺ ILC2s in the peripheral blood of patients with asthma. (A) Representative results of intracellular staining of IL-13 expression in ILC2s in patients with asthma and healthy control subjects. (B) IL-13⁺ ILC2s' proportion in patients with asthma with different control status. * $P < 0.01$ was considered a significant difference.

reversible airway obstruction. That being said, asthma is more likely a syndrome, on the basis that the increasing heterogeneity of patients with asthma becomes more apparent as additional subsets of patients are

defined. It remains unclear whether the heterogeneity of asthma phenotypes reflects the activation of different contributory pathways or indeed similar pathways simply being influenced by gene–environment

interactions, resulting in dissimilar phenotypes.

Growing evidence suggests that ILC2s may play critical roles in the pathogenesis of asthma, because these cells link both the innate and the adaptive immune responses within the hypersensitive airway. As the first natural barrier, the airway epithelium is responsible for protecting the body from external antigens including allergens, viruses, and foreign proteins. IL-25, IL-33, and TSLPs are the major cytokines secreted by airway epithelial cells after a defensive trigger. Several research groups have already demonstrated that ILC2s can be activated through the stimulation of IL-25, IL-33, and TSLP (14, 15, 29), because their receptors are expressed on the surface of ILC2s. After activation, ILC2s participate in the initiation of Th2 cell–mediated allergic lung inflammation by recruiting dendritic cells into the draining lymph nodes and inducing Th2 polarization in a contact-dependent manner (major histocompatibility complex class II and T-cell receptor). ILC2s also contribute to airway hyperreactivity, mucus overproduction, and smooth muscle constriction, independent of adaptive immunity because of the large amount of IL-13 production. IL-5 produced by ILC2s will also likely aid eosinophil homeostasis (18, 19). Finally, ILC2s have also been shown to be required in influenza-induced AHR by connecting the IL-33/IL-13 axis (30).

The “asthma control status” is defined by the effects of asthma that can be observed in patients or that have been reduced or removed by treatments; they include clinical symptom control and risk factors for future poor outcome (1–3). According to clinical symptom control, asthma can be divided into three control categories: uncontrolled, partly controlled, and well controlled. Uncontrolled asthma may develop as a consequence of several factors such as first-initiated asthma, improper inhaler technique (31), improper medication, poor medication adherence (32), incorrect diagnosis of asthma with symptoms caused by alternative conditions, and so on. Better understanding of the cellular and molecular mechanisms within asthmas with different control status helps us identify new therapeutic targets for potentially more effective treatments.

Herein, we have demonstrated a strong linkage between activated ILC2s in the

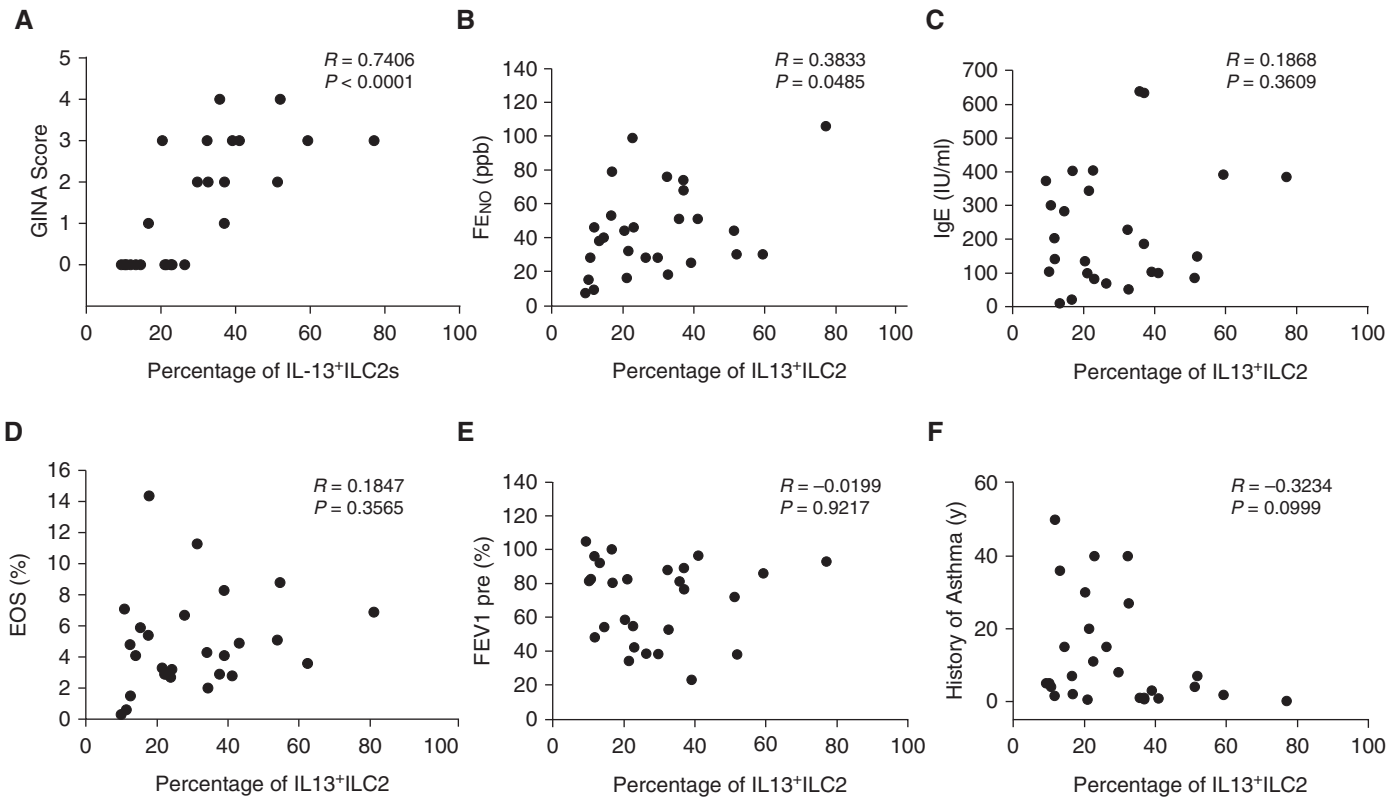


Figure 4. Correlation analysis between IL-13⁺ILC2s and clinical parameters. (A) Global Initiative for Asthma (GINA) score. (B) Fractional exhaled nitric oxide (FENO). (C) IgE levels. (D) Percentage of eosinophils (EOS). (E) Percentage of FEV₁ compared with predicted level (pre). (F) Years of asthma.

periphery and asthma control status. The ILC2 percentage in PBMCs has been shown to be significantly increased in individuals with allergic asthma compared with healthy control subjects from several groups (25–27). Our data highlight a similar phenomenon through testing of patients with asthma recruited from a top-tier

hospital in China. However, this difference was diminished when the patients were grouped according to control status. We also investigated the type 2 cytokine-producing capacities of ILC2s. Samples from patients with uncontrolled asthma and partly controlled asthma had significantly higher percentages of

IL-13⁺ILC2s compared with samples from the well-controlled asthma and healthy control groups. No differences were observed in IL-5⁺ILC2 percentages among different control status groups.

We not only compared the percentages of IL-13⁺ILC2s in different patients with asthma, but also compared them in the individuals from the uncontrolled groups before and after effective treatments. Strikingly, the IL-13⁺ILC2 percentages were decreased dramatically when their symptoms were well controlled. Taken together, these results demonstrate that the total number of peripheral ILC2s may be an indicator for distinguishing those with asthma from healthy control subjects, and moreover, that the percentages of IL-13⁺ILC2s might serve as a reliable predictor of asthma control status. The site of action of ILC2s is clearly localized to the airways. However, the relationship between levels of ILC2 cells within the circulation and lung tissues from different asthmatic control subgroups needs to be studied further. As of now, the physiological function of IL13⁺ILC2 within the circulation is unknown. Our

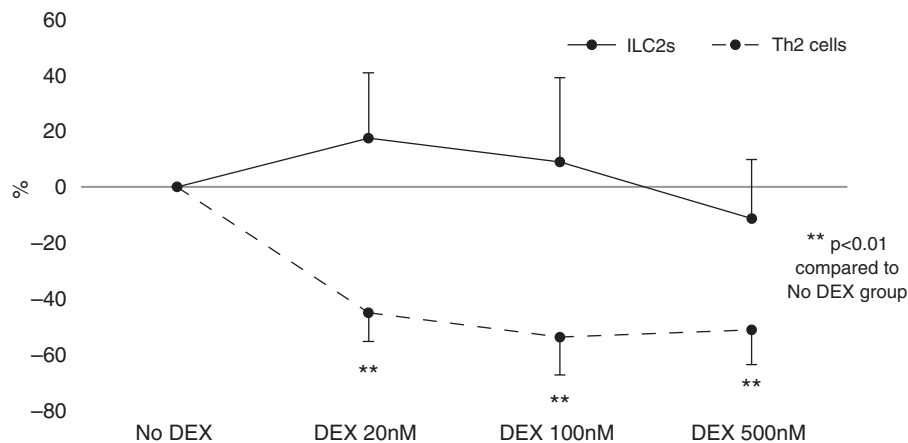


Figure 5. Inhibitory effects of dexamethasone (DEX) on activated ILC2s and CD4⁺ T-helper type 2 (Th2) cells *in vitro*. ILC2s and CD4⁺ Th2 cell functions were represented by IL-13 production. Doses of DEX were 20, 100, and 500 nM. Data are representative of results from six independent experiments.

initial attempts to identify ILC2s from sputum were not successful. This may have been because of a lack of adequate technique.

In the clinic, several examinations, including lung function, FE_{NO} , eosinophil/neutrophil counts, and serum IgE level, were performed routinely to guide treatments. The correlation analyses showed that IL-13⁺ILC2s were highly associated with the GINA scores calculated using the 2014 GINA guidelines. FE_{NO} level, an indicator of airway local inflammation, had a lower correlation with IL-13⁺ILC2s. Therefore, the incidence of these cells may provide us with a surrogate marker of the inflammatory status of the disease. Eosinophil numbers were confirmed to correlate with IL-5 levels, but not with IL-13 levels, which could explain the relationship between the eosinophil number and IL-13⁺ILC2s. In our study, no correlation between lung function and IL-13⁺ ILC2s was observed.

Kabata and colleagues demonstrated that DEX treatment did not suppress the accumulation of lung ILC2s and type 2 cytokine production in the bronchial epithelium in a mouse OVA + IL-33 model. In addition, *in vitro* proliferation of mouse ILC2s under IL-2/IL-33 was resistant to DEX treatment (24). In our study, we compared the effects of DEX on activated human ILC2s and CD4⁺ Th2 cells derived from PBMCs *in vitro*. Activated ILC2s were more resistant to DEX than were Th2 cells, revealing the potential role of ILC2 in glucocorticoid (GC)-resistant asthma. Further mechanistic study of GC resistance of activated ILC2 in human is needed.

One approach is to compare ILC2s' transcriptome in GC-sensitive and GC-insensitive patients. Robinette and colleagues have already shown the transcriptome profile from mouse ILCs (33); however, ILC2s' gene profile from human patients with asthma is still lacking,

both in stable disease and during exacerbation.

Conclusions

The emerging single-cell sequencing technology (34, 35) is a promising technology for identifying novel genes and their relevant signaling pathways within ILC2s in response to treatment. Analysis of these data may suggest potential new drug targets and biomarkers for poorly controlled asthma. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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