Allergology International 66 (2017) 302–309

Contents lists available at ScienceDirect

Allergology International

journal homepage: http://www.elsevier.com/locate/alit

Original article

Circulating activated innate lymphoid cells and mucosal-associated invariant T cells are associated with airflow limitation in patients with asthma

Ayako Ishimori [a, h], Norihiro Harada [a, b, c, **, h], Asako Chiba [d], Sonoko Harada [a, b], Kei Matsuno [a], Fumihiko Makino [a], Jun Ito [a], Shoichiro Ohta [e], Junya Ono [f], Ryo Atsuta [a], Kenji Izuhara [g], Kazuhisa Takahashi [a], Sachiko Miyake [d, *]

[a] Department of Respiratory Medicine, Juntendo University Faculty of Medicine and Graduate School of Medicine, Tokyo, Japan
[b] Research Institute for Diseases of Old Ages, Juntendo University Faculty of Medicine and Graduate School of Medicine, Tokyo, Japan
[c] Atopy (Allergy) Research Center, Juntendo University Faculty of Medicine and Graduate School of Medicine, Tokyo, Japan
[d] Department of Immunology, Juntendo University Faculty of Medicine and Graduate School of Medicine, Tokyo, Japan
[e] Department of Laboratory Medicine, Saga Medical School, Saga, Japan
[f] Shino-Test Corporation, Kanagawa, Japan
[g] Division of Medical Biochemistry, Department of Biomolecular Sciences, Saga Medical School, Saga, Japan

ARTICLE INFO

Article history:
Received 24 February 2016
Received in revised form
20 June 2016
Accepted 4 July 2016
Available online 27 August 2016

Keywords:
Airflow limitation
Asthma
Innate lymphoid cell
Mucosal-associated invariant T cell
Natural killer cell

Abbreviations:
ACT, asthma control test; ALX, low-frequency reactance area; COPD, chronic obstructive pulmonary disease; FeNO, fractional exhaled nitric oxide; FEV_1%, forced expiratory volume in 1 s/forced vital capacity; FEV_1, forced expiratory volume in 1 s; FOT, forced oscillation technique; Fres, resonant frequency; FVC, forced vital capacity; GINA, Global Initiative for Asthma; IFN-γ, interferon γ; IL, interleukin; ILC, innate lymphoid cell; ILC1, group 1 innate lymphoid cell; ILC2, group 2 innate lymphoid cell;

ABSTRACT

Background: A variety of innate subsets of lymphoid cells such as natural killer (NK) cells, several populations of innate lymphoid cells (ILCs), and mucosal-associated invariant T (MAIT) cells as innate-like T lymphocytes are involved in asthma and may have important effector functions in asthmatic immune responses. In the present study, we investigated whether NK cells, ILCs, and MAIT cells in the peripheral blood of patients with asthma would be associated with clinical asthma parameters.
Methods: We recruited 75 adult patients with mild to severe asthma. The peripheral blood mononuclear cells in peripheral venous blood samples from the patients were purified and stained with different combinations of appropriate antibodies. The cells were analyzed by flow cytometry.
Results: The percentage of activated (i.e., $CD69^+$) NK cells in the total NK cell population was negatively correlated with FEV_1% which is calculated by the forced expiratory volume in 1 s (FEV_1)/the forced vital capacity (FVC). The percentages of $CD69^+$ ILC1s and ILC2s were negatively correlated with FEV_1% and % FEV_1. The percentage of $CD69^+$ ILC3s was positively correlated with BMI, and the percentage of $CD69^+$ MAIT cells was negatively correlated with FEV_1%. Moreover, the percentage of $CD69^+$ NK cells, ILC1s, ILC2s, ILC3s, and MAIT cells were positively correlated with each other.
Conclusions: For the first time, our data showed that activated NK cells, ILC1s, ILC2s, ILC3s, and MAIT cells were positively correlated with each other and may be associated with airflow limitation in patients with asthma.

* Corresponding author. Department of Immunology, Juntendo University Faculty of Medicine and Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan.
** Corresponding author. Department of Respiratory Medicine, Juntendo University Faculty of Medicine and Graduate School of Medicine, 3-1-3 Hongo, Bunkyo-ku, Tokyo 113-8431, Japan.
E-mail addresses: s-miyake@juntendo.ac.jp (S. Miyake), nor@juntendo.ac.jp (N. Harada)
Peer review under responsibility of Japanese Society of Allergology.
[h] AI and NH contributed equally to this project and are considered co-first authors.

http://dx.doi.org/10.1016/j.alit.2016.07.005

ILC3, group 3 innate lymphoid cell; MAIT, mucosal-associated invariant T; NK, natural killer; PBMC, peripheral blood mononuclear cells; PEF, peak expiratory flow; R20, respiratory resistance at 20 Hz; R5, respiratory resistance at 5 Hz; TCR, T cell antigen receptor; X5, respiratory reactance at 5 Hz

Introduction

Allergic asthma is characterized by chronic airway inflammation, increased mucus production in the bronchioles, and airway hyperreactivity to a variety of specific and nonspecific stimuli. Several cell types are involved in airway inflammation; however, acquired immunity and antigen-specific Th2 cells, which secrete Th2 cytokines such as interleukin (IL)-4, IL-5, and IL-13 may drive asthma pathobiology.[1–4] The concentration of Th2 cytokines appears to be correlated with the severity of disease. These cytokines are responsible for the recruitment and activation of other cell types such as eosinophils, which have been associated with lung injury. The Th2 immune responses also mediate the production of mucus by the airway epithelium, which contributes to airway obstruction that constitutes a major component of the pathology of allergic airway inflammation.[5–7] In contrast to the enormous receptor diversity of T cell antigen receptors (TCRs) on conventional T cells, several lymphocyte subpopulations with limited repertoire diversity have been identified such as mucosal-associated invariant T (MAIT) cells.[8,9] The MAIT cells constitute an abundant population of innate-like T cells that express a semi-invariant TCR and have been reported to be involved in various disease conditions including infections and autoimmune diseases.[10–12]

The “innate” subsets of lymphoid cells are divided into innate-like T lymphocytes, which express a semi-invariant TCR, and innate lymphoid cells (ILCs), which lack TCRs.[13] The ILCs also lack myeloid cell markers and dendritic cell phenotypical markers.[13] Several distinct ILC populations have recently been identified that are similar to natural killer (NK) cells, which are the prototypical ILC population. Several reports have been proposed to classify ILC populations based on their phenotypical and functional characteristics.[13,14] The nomenclature is based on helper T cell nomenclature and categorizes the ILC populations into three groups: (1) group 1 ILCs (ILC1s), which consists of ILCs that produce interferon γ (IFN-γ); (2) group 2 ILCs (ILC2s), which produces type 2 cytokines—in particular IL-5 and IL-13; and (3) group 3 ILCs (ILC3s), which produce Il-17 and/or IL-22. In this model, NK cells are classified as ILC1s.[13,14] Studies have highlighted many roles for ILCs and innate-like T cells in regulating the immune system. The cells are heterogeneous in their tissue location, cytokine production, and effector functions.[15–18]

Mucosal-associated invariant T cells are abundant in human peripheral blood where they usually represent 1–10% of the total αβ T cell population. These cells also exist in the gut mucosa, liver, and lung.[9,19–24] The ILC2s were also initially described in gut and lung, and ILC2s have been detected in human peripheral blood from healthy and asthmatic people.[25–29] Thus, in the present study, we investigated whether the frequency of NK cells, ILCs, and MAIT cells in peripheral blood in patients with asthma was associated with clinical asthma parameters.

Methods

Patients

The patients were recruited from our outpatient clinic at Juntendo University Hospital (Tokyo, Japan). Patients were enrolled who had mild to severe asthma and were aged 20 years or older. Asthma was diagnosed by a compatible clinical history of episodic symptoms with airflow limitation and by variations in pulmonary function monitored by forced expiratory volume in 1 s (FEV_1) or peak expiratory flow (PEF) in accordance with the Global Initiative for Asthma (GINA) guidelines.[30] The severity of disease in eligible patients was also assessed in accordance with the GINA guidelines.[30] The present study was reviewed and approved by the Juntendo University Research Ethics Committee (Tokyo, Japan). Written informed consent was obtained from each patient before their participation in the study. This study was registered in the UMIN Clinical Trial Registry (UMIN000009968) on February 5, 2013 (http://www.umin.ac.jp/).

Patients having any of the following criteria were excluded: a diagnosis of chronic obstructive pulmonary disease (COPD), as defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines, and any current respiratory disorder other than asthma.[31]

The asthma control test (ACT) score; pulmonary function tests, which includes respiratory resistance and reactance by the forced oscillation technique (FOT); and the fractional exhaled nitric oxide (FeNO) levels were measured. The FeNO levels were measured in accordance with the American Thoracic Society recommendations at a constant flow of 0.05 L/s against an expiratory resistance of 20 cm water with a chemiluminescence analyzer (NOA 280i; Sievers, Boulder, CO, USA). The FOT was measured using the MostGraph-01 FOT device (Chest MI, Tokyo, Japan).

Flow cytometry

Peripheral venous blood samples were collected in heparin-containing tubes and plasma was frozen at −80 °C immediately after centrifugation for the measurement of periostin levels. Peripheral blood mononuclear cells (PBMCs; at 3×10^6/well) were purified by density-gradient centrifugation using Ficoll–Paque Plus solution (GE Healthcare, Tokyo, Japan). The cells were stained with different combinations of appropriate antibodies for 30 min at 4 °C. The following surface marker antibodies were used in this study: anti-CD11c-FITC (BioLegend, San Diego, CA, USA), anti-CD14-FITC (BioLegend), anti-CD1a-FITC (BioLegend), anti-CD19-FITC (BD Biosciences, San Jose, CA, USA), anti-CD34-FITC (BioLegend), anti-TCR-Pan-γδ-FITC (Beckman Coulter, Miami, FL, USA), anti-CD123-FITC (BioLegend), anti-BDCA2-FITC (BioLegend), anti-FCεR1-FITC (BioLegend), anti-CD3-APC-H7 (BD Biosciences), anti-Vα7.2-PE (BioLegend), anti-CD161-PerCPCy5.5 (BioLegend), anti-CD56-Alexa Fluor 700 (BD Biosciences), anti-CD69-APC (BioLegend), anti-CD294

Table 1
Baseline characteristics of the study population.

	Total n = 75	Mild to moderate asthma n = 27	Severe asthma n = 48	*P* value
Sex (M/F), n (%)	30 (40.0)/45 (60.0)	17 (63.0)/10 (37.0)	13 (27.1)/35 (72.9)	0.025*
Age (y)	54.09 ± 16.73	59.00 ± 15.79	51.33 ± 16.76	0.056
Age at asthma onset (y)	39.57 ± 22.71	44.37 ± 21.85	36.88 ± 22.97	0.159
Body mass index (kg/cm^2)	23.90 ± 4.87	23.44 ± 3.65	24.16 ± 5.46	0.950
Smoking history (never/ex/current), n (%)	43 (57.3)/29 (38.7)/3 (4.0)	10 (37.0)/15 (55.6)/2 (7.4)	33 (68.8)/14 (29.2)/1 (2.1)	0.025*
Atopic dermatitis, n (%)	19 (25.3)	6 (22.2)	13 (27.1)	0.089
Allergic rhinitis, n (%)	49 (65.3)	16 (59.3)	33 (68.8)	0.455
Daily dose of inhaled corticosteroids (FP equivalent dose, μg)	554.67 ± 404.38	151.85 ± 96.56	781.25 ± 326.59	<0.001*
Daily dose of oral corticosteroids, n (PSL equivalent dose, mg)	5 (0.24 ± 1.29)	0 (0.00 ± 0.00)	5 (0.38 ± 1.61)	0.153
Asthma control test (ACT) score	23.42 ± 2.81	24.85 ± 0.46	22.60 ± 3.24	<0.001*
FVC (L)	3.32 ± 1.02	3.59 ± 1.01	3.16 ± 1.00	0.076
%FVC (%)	103.28 ± 17.42	106.47 ± 16.52	101.50 ± 17.83	0.221
FEV_1 (L)	2.48 ± 0.84	2.56 ± 0.79	2.43 ± 0.86	0.522
%FEV_1 (%)	91.92 ± 19.19	91.18 ± 17.17	92.33 ± 20.40	0.585
FEV_1% (%)	74.44 ± 10.17	71.04 ± 7.36	76.35 ± 11.06	0.002*
PEF (L/s)	7.43 ± 2.00	7.93 ± 2.16	7.15 ± 1.87	0.106
%PEF (%)	103.49 ± 20.44	103.28 ± 18.05	103.61 ± 21.86	0.946
R5 (cmH_2O/L/s)	3.45 ± 1.34	3.43 ± 1.39	3.47 ± 1.32	0.915
R20 (cmH_2O/L/s)	2.90 ± 1.03	2.84 ± 1.05	2.94 ± 1.03	0.690
X5 (cmH_2O/L/s)	−0.69 ± 1.07	−0.94 ± 1.57	−0.55 ± 0.60	0.812
ALX (cmH_2O/L/s)	4.19 ± 9.08	6.69 ± 14.06	2.78 ± 3.85	0.761
Fres (Hz)	8.38 ± 3.89	8.90 ± 4.91	8.08 ± 3.20	0.593
FeNO (ppb)	59.60 ± 48.48	68.95 ± 55.80	54.34 ± 43.57	0.046*
Peripheral eosinophils (/μL)	242.49 ± 213.63	253.30 ± 203.79	236.41 ± 220.85	0.512
Peripheral neutrophils (/μL)	3913.03 ± 1558.43	3284.17 ± 898.65	4266.77 ± 1738.45	0.016*
Serum IgE (IU/mL)	469.48 ± 1314.49	496.45 ± 722.38	454.31 ± 1559.23	0.083
Serum periostin (ng/mL)	89.36 ± 33.19	95.63 ± 26.54	85.83 ± 36.19	0.064

The data are presented as the mean ± the standard deviation, unless otherwise indicated.
Abbreviations for all tables: ACT, asthma control test; ALX, low-frequency reactance area; FeNO, fractional exhaled nitric oxide; FEV_1%, forced expiratory volume in 1 s/forced vital capacity; FEV_1, forced expiratory volume in 1 s; Fres, resonant frequency; FVC, forced vital capacity; ILC, innate lymphoid cell; ILC1, group 1 innate lymphoid cell; ILC2, group 2 innate lymphoid cell; ILC3, group 3 innate lymphoid cell; MAIT, mucosal-associated invariant T; NA, not applicable; NK, natural killer; PEF, peak expiratory flow; R20, respiratory resistance at 20 Hz; R5, respiratory resistance at 5 Hz; SE, standard error; X5, respiratory reactance at 5 Hz.
*Indicates $p < 0.05$, mild to moderate asthma versus severe asthma.

(CRTH2)-Brilliant Violet 421 (BioLegend), anti-CD127-Brilliant Violet 605 (BioLegend), and anti-CD117 (c-Kit)-PE-CF594 (BD Biosciences). Lineage marker negative (Lin^-) was defined as $CD1a^-$, $CD3^-$, $CD11c^-$, $CD14^-$, $CD19^-$, $CD34^-$, $TCR\gamma\delta^-$, $CD123^-$, $BDCA2^-$, and $FC\varepsilon R1^-$. Mucosal-associated invariant T cells were identified as $CD3^+$ $V\alpha7.2$ TCR^+ $CD161^{high}$ cells; NK cells, as $CD3^-$ $CD56^+$ cells; ILC1, as Lin^- $CD127^+$ $CD161^+$ $CD117^-$ $CRTH2^-$ cells; ILC2, as Lin^- $CD127^+$ $CD161^+$ $CRTH2^+$ cells; and ILC3, as Lin^- $CD127^+$ $CD161^+$ $CD117^+$ $CRTH2^-$ cells. The activation marker CD69 was analyzed in total MAIT cells, NK cells, and ILCs. After overnight fixation, the cells were analyzed by the fluorescence-activated cell sorting (FACS) LSRFortessa cell analyzer (BD Biosciences). The FACS data were analyzed with Flowjo software (Version 7.6.5; Tree Star, Ashland, OR, USA).

Quantification of the periostin serum level

The sera of patients were collected by density-gradient centrifugation of blood samples and frozen at −80 °C. Periostin levels were measured with an enzyme-linked immunosorbent assay (Shino-Test, Kanagawa, Japan), as described previously.[32]

Statistical analysis

Sample normality was examined using the D'Agostino and Pearson test. Differences in parameters between populations were analyzed for significance using the Student *t* test, Mann–Whitney *U* test, analysis of variance, and Kruskal–Wallis test, as needed. For

Table 2
The circulating innate subsets of lymphoid cells in patients with asthma.

	Total n = 75	Mild to moderate asthma n = 27	Severe asthma n = 48	*P* value
NK cells (% of lymphocytes)	11.11 ± 6.37	10.08 ± 5.42	11.69 ± 6.83	0.406
ILC1 (% of Lin^- $CD127^+$ $CD161^+$ cells)	61.48 ± 12.88	61.97 ± 12.50	61.21 ± 13.22	0.574
ILC2 (% of Lin^- $CD127^+$ $CD161^+$ cells)	24.39 ± 12.75	22.20 ± 12.79	25.62 ± 12.69	0.163
ILC3 (% of Lin^- $CD127^+$ $CD161^+$ cells)	14.13 ± 5.97	15.83 ± 5.79	13.17 ± 5.91	0.064
MAIT cells (% of $CD3^+$ cells)	2.21 ± 2.10	2.01 ± 1.66	2.32 ± 2.32	0.932
$CD69^+$ NK cells (% of NK cells)	19.69 ± 6.60	20.02 ± 6.25	19.51 ± 6.85	0.537
$CD69^+$ ILC1 (% of ILC1)	17.67 ± 6.61	17.10 ± 4.77	17.99 ± 7.48	0.778
$CD69^+$ ILC2 (% of ILC2)	27.60 ± 7.99	26.36 ± 5.74	28.30 ± 8.99	0.859
$CD69^+$ ILC3 (% of ILC3)	28.45 ± 14.42	26.62 ± 7.99	29.48 ± 17.10	0.761
$CD69^+$ MAIT cells (% of MAIT cells)	27.51 ± 13.70	31.88 ± 15.28	25.05 ± 12.21	0.037*

*Indicates $p < 0.05$.
The data are presented as the mean ± the standard deviation, unless otherwise indicated.

correlation between variables, Pearson's correlation coefficient and Spearman's rank correlation coefficient were used where appropriate. Differences were statistically significant when the p values were 0.05 or less. Statistical analyses were performed by version 6 of Graph Pad Prism software (GraphPad Software, La Jolla, CA, USA). The relationships between the parameters and the MAIT cells were assessed by age-adjusted and unadjusted regression analysis using STATA12.1 software (STATA Corporation LP, College Station, TX, USA).

Results

Baseline characteristics

Seventy-five patients with asthma were enrolled in this study. The baseline characteristics of eligible patients are summarized in Table 1. The male to female ratio was 30:45, and the mean age was 54.09 ± 16.73 years. The mean duration of asthma was 14.5 ± 15.1 years, and the mean FEV_1% which is calculated by FEV_1/the forced vital capacity (FVC) was 74.44% ± 10.17%. Table 1 shows the patients' baseline characteristics separated by the severity of asthma, which was assessed in accordance with the GINA guidelines.[30] Forty eight patients with severe asthma were compared to 27 patients who had mild to moderate asthma. The male to female ratio, the ACT score, and the FeNO levels in the severe asthmatic group were significantly lower, whereas the never-smoker/current and ex-smoker ratio, the daily dose of inhaled corticosteroids, the FEV_1%, and the peripheral neutrophil counts in the severe asthmatic group were significantly higher.

Circulating innate subsets of lymphoid cells in patients with asthma

We examined the frequency of ILCs and MAIT cells in the peripheral blood of patients with asthma by using flow cytometry.

Table 4
The correlation between circulating MAIT cells, after adjusting for age.

	Log MAIT cells					
	Single			Age-adjusted		
	β	SE	*P* value	β	SE	*P* value
Age (y)	−0.014	0.003	<0.001*	NA	NA	NA
Age at asthma onset (y)	−0.008	0.002	0.001*	0.000	0.003	0.899
BMI (kg/cm^2)	−0.028	0.010	0.008*	−0.027	0.009	0.003*
Daily dose of inhaled corticosteroids (FP equivalent dose, μg)	−0.000	0.000	0.781	−0.000	0.000	0.331
Daily dose of oral corticosteroids (PSL equivalent dose, mg)	−0.022	0.036	0.546	−0.010	0.031	0.741
ACT (score)	0.009	0.019	0.651	0.011	0.016	0.510
FVC (L)	0.186	0.047	<0.001*	0.080	0.052	0.133
%FVC (%)	0.002	0.003	0.530	0.001	0.003	0.632
FEV_1% (%)	0.007	0.005	0.202	−0.005	0.005	0.334
FEV_1 (L)	0.236	0.057	<0.001*	0.076	0.073	0.301
%FEV_1 (%)	−0.000	0.003	0.882	−0.002	0.002	0.488
PEF (L/s)	0.095	0.024	<0.001*	0.047	0.025	0.065
%PEF (%)	−0.001	0.003	0.583	0.001	0.002	0.699
R5 (cmH_2O/L/s)	−0.091	0.382	0.020*	−0.059	0.034	0.082
R20 (cmH_2O/L/s)	−0.107	0.050	0.036*	−0.084	0.043	0.054
X5 (cmH_2O/L/s)	0.069	0.049	0.166	−0.035	0.047	0.460
ALX (cmH_2O/L/s)	−0.004	0.006	0.452	0.008	0.005	0.163
Fres (Hz)	−0.034	0.013	0.012*	−0.008	0.013	0.512
FeNO (ppb)	0.000	0.001	0.772	0.000	0.001	0.970
Peripheral eosinophils (/μL)	−0.000	0.000	0.338	−0.000	0.000	0.500
Peripheral neutrophils (/μL)	−0.000	0.000	0.760	−0.000	0.000	0.355
Serum IgE (IU/mL)	−0.000	0.000	0.854	−0.000	0.000	0.330
Serum periostin (ng/mL)	−0.004	0.002	0.006*	−0.003	0.001	0.067
NK cells (% of lymphocytes)	0.002	0.008	0.813	0.006	0.007	0.419
ILC1 (% of Lin$^-$ CD127$^+$ CD161$^+$ cells)	−0.005	0.004	0.212	−0.006	0.003	0.105
ILC2 (% of Lin$^-$ CD127$^+$ CD161$^+$ cells)	0.006	0.004	0.173	0.005	0.003	0.124
ILC3 (% of Lin$^-$ CD127$^+$ CD161$^+$ cells)	−0.002	0.009	0.833	0.002	0.008	0.833

*Indicates $p < 0.05$.

Table 3
The correlation between the circulating innate subsets of lymphoid cells.

	NK cells		ILC1		ILC2		ILC3		MAIT cells	
	r	*P* value	r	*P* value	r	*P* value	r	*P* value	r	*P* value
Age (y)	0.135	0.250	−0.044	0.707	0.007	0.952	0.103	0.381	−0.528	<0.001*
Age at asthma onset (y)	0.167	0.154	−0.061	0.603	0.029	0.802	0.048	0.680	−0.385	<0.001*
BMI (kg/cm^2)	0.044	0.706	−0.089	0.448	0.174	0.136	−0.262	0.023*	−0.265	0.022*
Daily dose of inhaled corticosteroids (FP equivalent dose, μg)	−0.024	0.836	−0.025	0.829	0.081	0.491	−0.110	0.347	−0.028	0.810
Daily dose of oral corticosteroids (PSL equivalent dose, mg)	0.008	0.947	0.329	0.004*	−0.250	0.031*	−0.248	0.032*	−0.078	0.504
ACT (score)	−0.083	0.483	−0.172	0.143	0.101	0.392	0.239	0.040*	−0.049	0.680
FVC (L)	−0.030	0.798	−0.141	0.228	0.049	0.679	0.173	0.139	0.408	<0.001*
%FVC (%)	−0.269	0.020*	−0.119	0.310	−0.024	0.839	0.261	0.024*	0.105	0.371
FEV_1% (%)	−0.060	0.609	−0.005	0.963	0.083	0.480	−0.177	0.130	0.196	0.093
FEV_1 (L)	−0.063	0.590	−0.135	0.248	0.081	0.491	0.079	0.503	0.474	<0.001*
%FEV_1 (%)	−0.247	0.033*	−0.093	0.425	0.017	0.885	0.103	0.379	0.036	0.762
PEF (L/s)	0.025	0.832	−0.168	0.149	0.100	0.395	0.201	0.084	0.450	<0.001*
%PEF (%)	−0.195	0.093	−0.086	0.464	0.003	0.978	0.159	0.174	−0.050	0.670
R5 (cmH_2O/L/s)	0.043	0.714	0.094	0.425	−0.065	0.579	−0.047	0.692	−0.259	0.025*
R20 (cmH_2O/L/s)	0.017	0.883	0.102	0.386	−0.083	0.479	−0.080	0.494	−0.249	0.031*
X5 (cmH_2O/L/s)	−0.090	0.442	−0.159	0.174	0.112	0.338	0.094	0.424	0.342	0.003*
ALX (cmH_2O/L/s)	0.098	0.404	0.156	0.180	−0.115	0.326	−0.074	0.526	−0.358	0.002*
Fres (Hz)	0.117	0.318	0.157	0.178	−0.120	0.307	−0.076	0.516	−0.327	0.004*
FeNO (ppb)	0.195	0.094	0.102	0.385	−0.089	0.449	0.001	0.995	−0.078	0.504
Peripheral eosinophils (/μL)	0.149	0.202	−0.087	0.459	0.177	0.129	−0.130	0.268	−0.161	0.167
Peripheral neutrophils (/μL)	0.105	0.371	0.141	0.228	−0.080	0.494	−0.116	0.323	0.048	0.681
Serum IgE (IU/mL)	0.070	0.553	0.202	0.083	−0.180	0.123	−0.026	0.822	−0.059	0.616
Serum periostin (ng/mL)	0.117	0.320	−0.002	0.984	0.006	0.959	0.065	0.578	−0.301	0.009*
NK cells (% of lymphocytes)	NA	NA	−0.047	0.690	0.166	0.156	−0.195	0.093	0.062	0.595
ILC1 (% of Lin$^-$ CD127$^+$ CD161$^+$ cells)	−0.047	0.690	NA	NA	−0.881	<0.001*	−0.254	0.028*	−0.140	0.231
ILC2 (% of Lin$^-$ CD127$^+$ CD161$^+$ cells)	0.166	0.156	−0.881	<0.001*	NA	NA	−0.155	0.184	0.154	0.186
ILC3 (% of Lin$^-$ CD127$^+$ CD161$^+$ cells)	−0.195	0.093	−0.254	0.028*	−0.155	0.184	NA	NA	−0.028	0.814
MAIT cells (% of CD3$^+$ cells)	0.062	0.595	−0.140	0.231	0.154	0.186	−0.028	0.814	NA	NA

*Indicates $p < 0.05$.

The gating strategy for PBMCs is shown in Supplementary Figure 1. In this study, the frequencies of NK cells and MAIT cells were demonstrated by the ratio of $CD3^-$ $CD56^+$ cells to lymphocytes and the ratio of $CD3^+$ Vα7.2 TCR^+ $CD161^{high}$ cells to $CD3^+$ cells, respectively. The frequencies of ILC1s, ILC2s, and ILC3s were also shown by the ratio of Lin^- $CD127^+$ $CD161^+$ $CD117^-$ $CRTH2^-$ cells to Lin^- $CD127^+$ $CD161^+$ cells, Lin^- $CD127^+$ $CD161^+$ $CRTH2^+$ cells to Lin^- $CD127^+$ $CD161^+$ cells, and Lin^- $CD127^+$ $CD161^+$ $CD117^+$ $CRTH2^-$ cells to Lin^- $CD127^+$ $CD161^+$ cells, respectively. The frequencies of NK cells (mean, 11.11% ± 6.37%), ILC1s (mean, 61.48% ± 12.88%), ILC2s (mean, 24.39% ± 12.75%), ILC3s (mean, 14.13% ± 5.97%), and MAIT cells (mean, 2.21% ± 2.10%) were detected in the peripheral blood of patients with asthma (Table 2). There were no significant differences between the asthma severity groups in the frequencies of NK cells, ILC1s, ILC2s, ILC3s, and MAIT cells (Table 2).

We next examined the cell surface expression of the activation marker CD69 on NK cells, ILCs, and MAIT cells. The expression of CD69, which is a member of the C-type lectin super-family, suggests the activation of each innate subset of lymphoid cells.[24,33,34] The percentages of $CD69^+$ NK cells, ILCs, and MAIT cells are shown in Table 2. The percentage of $CD69^+$ MAIT cells in the peripheral blood is lower in patients with severe asthma than in patients with mild to moderate asthma (Table 2). Human NK cells can be divided into two subsets: $CD56^{bright}$ NK cells (which produce immunoregulatory cytokines such as IFN-γ, IL-10, and IL-13) and $CD56^{dim}$ NK cells (which represent a fully mature highly cytotoxic NK cell subset).[35] There were also no significant differences between the asthma severity groups in the frequencies of $CD56^{bright}$ and $CD56^{dim}$ NK cells and the percentages of $CD69^+$ $CD56^{bright}$ and $CD56^{dim}$ NK cells (Supplementary Table 1).

Correlation between the circulating innate subsets of lymphoid cells

The frequency of NK cells was negatively correlated with percent predicted FVC (%FVC) and percent predicted FEV_1 (%FEV_1) (Table 3). The frequency of $CD56^{bright}$ NK cells was negatively correlated with age, percent predicted PEF (%PEF), low-frequency reactance area (ALX), and resonant frequency (Fres), and positively correlated with respiratory reactance at 5 Hz (X5) and the frequency of MAIT cells (Supplementary Table 2). The frequency of $CD56^{dim}$ NK cells was negatively correlated with %FVC and %FEV_1 and positively correlated with the frequency of total NK cells (Supplementary Table 2). The daily dose of oral corticosteroids was positively correlated with the frequency of ILC1s and negatively correlated with the frequencies of ILC2s and ILC3s (Table 3). The frequency of ILC1s was negatively correlated with the frequencies of ILC2s and ILC3s (Table 3). The frequency of ILC3s was negatively correlated with BMI and positively correlated with ACT score and % FVC (Table 3). The frequency of peripheral blood MAIT cells in asthmatics was adjusted for age using multiple regression analysis because the frequency of peripheral blood MAIT cells and the total peripheral blood MAIT cells reportedly decreases with age.[36,37] The frequency of peripheral blood MAIT cells in asthmatic patients was negatively correlated with age, the age of asthma onset, and airflow limitation, before adjusting for age using multiple regression

Table 5
The correlation between circulating $CD69^+$ innate subsets of lymphoid cells.

	$CD69^+$ NK cells		$CD69^+$ ILC1		$CD69^+$ ILC2		$CD69^+$ ILC3		$CD69^+$ MAIT cells	
	r	*P* value	r	*P* value	r	*P* value	r	*P* value	r	*P* value
Age (y)	−0.124	0.289	−0.102	0.384	−0.065	0.580	0.023	0.842	0.179	0.124
Age at asthma onset (y)	−0.123	0.294	−0.049	0.674	−0.033	0.781	0.034	0.773	0.112	0.337
BMI (kg/cm^2)	−0.033	0.780	0.101	0.390	0.164	0.160	0.275	0.017*	−0.055	0.641
Daily dose of inhaled corticosteroids (FP equivalent dose, μg)	−0.108	0.356	−0.023	0.847	0.066	0.572	0.018	0.879	−0.187	0.109
Daily dose of oral corticosteroids (PSL equivalent dose, mg)	−0.023	0.842	−0.031	0.790	0.149	0.201	0.026	0.824	−0.134	0.250
ACT (score)	0.090	0.446	0.122	0.300	−0.082	0.486	0.024	0.838	0.126	0.283
FVC (L)	0.275	0.017*	0.190	0.103	0.014	0.906	−0.005	0.964	0.100	0.393
%FVC (%)	0.062	0.600	−0.104	0.373	−0.137	0.241	0.006	0.961	−0.049	0.676
FEV_1% (%)	−0.242	0.037*	−0.316	0.006*	−0.236	0.041*	−0.149	0.202	−0.247	0.033*
FEV_1 (L)	0.170	0.144	0.065	0.578	−0.057	0.630	−0.050	0.668	0.013	0.910
%FEV_1 (%)	−0.154	0.188	−0.323	0.005*	−0.281	0.015*	−0.071	0.543	−0.157	0.178
PEF (L/s)	0.147	0.209	0.027	0.820	0.029	0.803	−0.044	0.709	−0.050	0.668
%PEF (%)	−0.280	0.015*	−0.431	<0.001*	−0.200	0.085	−0.094	0.421	−0.220	0.058
R5 ($cmH_2O/L/s$)	−0.090	0.445	−0.016	0.893	−0.026	0.827	0.033	0.777	−0.022	0.854
R20 ($cmH_2O/L/s$)	−0.083	0.480	−0.016	0.892	−0.023	0.847	0.007	0.950	−0.031	0.792
X5 ($cmH_2O/L/s$)	0.063	0.592	0.058	0.620	−0.070	0.552	−0.105	0.373	0.046	0.696
ALX ($cmH_2O/L/s$)	−0.074	0.526	−0.060	0.607	0.077	0.510	0.104	0.376	−0.060	0.607
Fres (Hz)	−0.042	0.722	−0.056	0.635	0.097	0.410	0.118	0.314	−0.068	0.560
FeNO (ppb)	0.059	0.615	0.201	0.084	0.110	0.350	0.006	0.956	0.116	0.321
Peripheral eosinophils (/μL)	−0.074	0.528	0.063	0.591	0.019	0.870	0.077	0.513	0.057	0.627
Peripheral neutrophils (/μL)	0.014	0.907	0.023	0.844	0.055	0.642	0.085	0.470	−0.058	0.623
Serum IgE (IU/mL)	0.121	0.301	0.148	0.205	0.102	0.385	0.001	0.992	0.132	0.259
Serum periostin (ng/mL)	−0.070	0.552	0.082	0.483	0.002	0.988	0.012	0.919	−0.042	0.720
NK cells (% of lymphocytes)	0.069	0.556	0.269	0.020*	0.183	0.116	0.013	0.909	0.121	0.303
ILC1 (% of Lin^- $CD127^+$ $CD161^+$ cells)	−0.203	0.081	−0.049	0.674	0.121	0.301	0.010	0.932	−0.087	0.458
ILC2 (% of Lin^- $CD127^+$ $CD161^+$ cells)	0.169	0.148	0.148	0.207	−0.040	0.731	0.171	0.142	0.087	0.456
ILC3 (% of Lin^- $CD127^+$ $CD161^+$ cells)	0.047	0.688	−0.226	0.052	−0.285	0.013*	−0.365	0.001*	−0.068	0.563
MAIT cells (% of $CD3^+$ cells)	0.147	0.209	0.079	0.499	−0.019	0.875	−0.059	0.612	−0.148	0.204
$CD69^+$ NK cells (% of NK cells)	NA	NA	0.686	<0.001*	0.424	<0.001*	0.376	<0.001*	0.468	<0.001*
$CD69^+$ ILC1 (% of ILC1)	0.686	<0.001*	NA	NA	0.589	<0.001*	0.628	<0.001*	0.503	<0.001*
$CD69^+$ ILC2 (% of ILC2)	0.424	<0.001*	0.589	<0.001*	NA	NA	0.468	<0.001*	0.218	0.060
$CD69^+$ ILC3 (% of ILC3)	0.376	<0.001*	0.628	<0.001*	0.468	<0.001*	NA	NA	0.274	0.018*
$CD69^+$ MAIT cells (% of MAIT cells)	0.468	<0.001*	0.503	<0.001*	0.218	0.060	0.274	0.018*	NA	NA

*Indicates $p < 0.05$.

analysis; however, there was no significant difference between the frequency of MAIT cells and age of asthma onset and airflow limitation, after adjusting for age (Table 4).

Correlation between circulating CD69$^+$-activated innate subsets of lymphoid cells

The percentage of CD69$^+$ NK cells in the total NK cells of patients with asthma was positively correlated with FVC and negatively correlated with FEV_1% and %PEF (Table 5). The percentage of CD69$^+$ ILC1s was negatively correlated with FEV_1%, $\%FEV_1$, and %PEF (Table 5). The percentage of CD69$^+$ ILC2s was also negatively correlated with FEV_1% and $\%FEV_1$ (Table 5). The percentage of CD69$^+$ ILC3s was positively correlated with BMI, and the percentage of CD69$^+$ MAIT cells was negatively correlated with FEV_1% (Table 5). The percentage of CD69$^+$ CD56^{bright} NK cells was negatively correlated with FEV_1%, $\%FEV_1$, and %PEF (Supplementary Table 3). The percentage of CD69$^+$ CD56^{dim} NK cells was positively correlated with FVC and serum IgE levels and negatively correlated with %PEF (Supplementary Table 3). The percentages of CD69$^+$ NK cells (including CD56^{bright} and CD56^{dim} NK cells), ILC1s, ILC2s, ILC3s, and MAIT cells in these total cells were positively correlated with each other except the correlation between CD69$^+$ ILC2s and MAIT cells (Table 5, Supplementary Table 3, Fig. 1). Moreover, even after adjusting for age using multiple regression analysis, the percentage of CD69$^+$ MAIT cells was correlated with %PEF, serum periostin levels, and with the percentage of CD69$^+$ NK cells, ILC1s, and ILC3s (Table 6). Furthermore, the percentage of CD69$^+$ MAIT cells was correlated with the percentage of CD69$^+$ ILC2s, after adjusting for age (Table 6). These findings suggested that circulating activated NK cells, ILC1s, ILC2s, ILC3s, and MAIT cells were positively correlated with each other and may be associated with airflow limitation in patients with asthma.

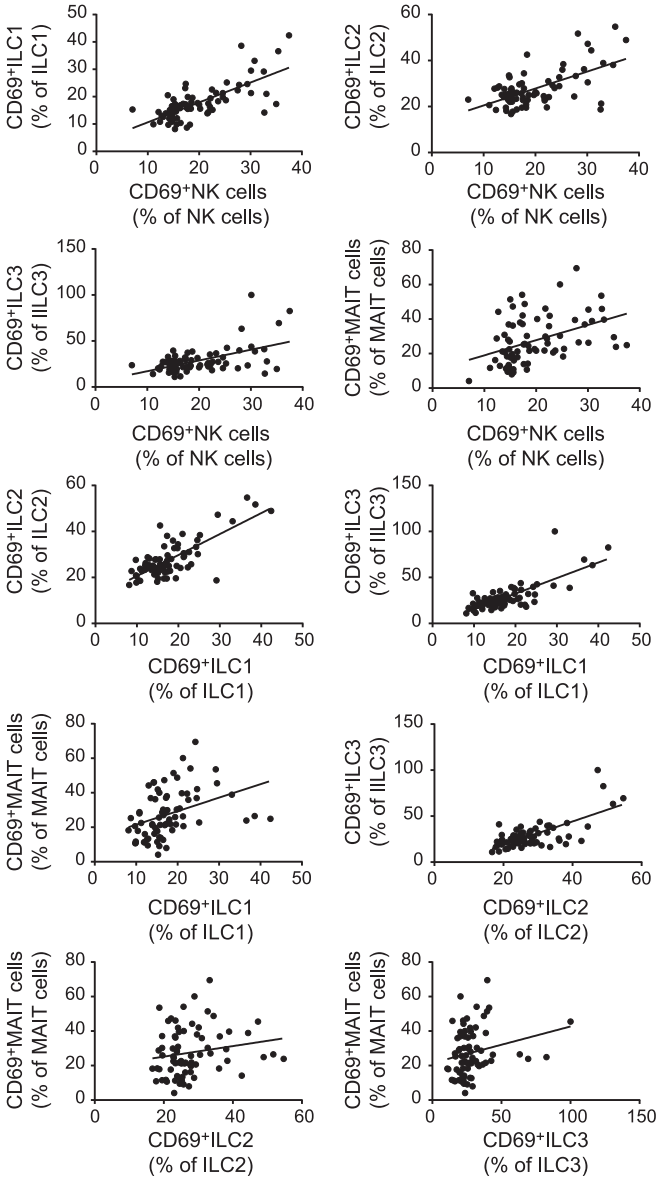

Fig. 1. The percentage of CD69$^+$ NK cells, ILC1s, ILC2s, ILC3s, MAIT cells positively correlated each other. ILC1, group 1 innate lymphoid cell; ILC2, group 2 innate lymphoid cell; ILC3, group 3 innate lymphoid cell; MAIT, mucosal-associated invariant T; NK, natural killer.

Discussion

To our knowledge, this is the first study to show that the CD69$^+$-activated NK cells, ILC1s, ILC2s, ILC3s, and MAIT cells were positively correlated with each other and associated with airflow limitation in patients with asthma. Asthma is a heterogeneous inflammatory disorder comprising distinct phenotypes, such as Th2-high asthma, neutrophilic asthma, viral-induced asthma, and obesity-associated asthma.[4,38] In group 1 ILCs, which include traditional NK cells and ILC1s, NK cells contribute to innate defense against viral infection

Table 6
The correlation between circulating CD69$^+$ MAIT cells, after adjusting for age.

	Log CD69$^+$ MAIT cells					
	Single			Age-adjusted		
	β	SE	*P* value	β	SE	*P* value
Age (y)	0.003	0.002	0.103	NA	NA	NA
Age at asthma onset (y)	0.001	0.001	0.416	−0.001	0.002	0.544
BMI (kg/cm^2)	−0.005	0.006	0.427	−0.005	0.006	0.394
Daily dose of inhaled corticosteroids (FP equivalent dose, μg)	−0.000	0.000	0.042*	−0.000	0.000	0.062
Daily dose of oral corticosteroids (PSL equivalent dose, mg)	−0.012	0.019	0.543	−0.014	0.019	0.463
ACT (score)	0.010	0.010	0.299	0.010	0.010	0.312
FVC (L)	0.017	0.027	0.535	0.059	0.031	0.065
%FVC (%)	−0.000	0.002	0.840	−0.000	0.002	0.901
FEV_1% (%)	−0.005	0.003	0.042*	−0.004	0.003	0.143
FEV_1 (L)	−0.003	0.330	0.924	0.061	0.044	0.169
$\%FEV_1$ (%)	−0.002	0.001	0.290	−0.001	0.001	0.360
PEF (L/s)	−0.005	0.014	0.710	0.007	0.016	0.635
%PEF (%)	−0.003	0.001	0.042*	−0.003	0.001	0.015*
FeNO (ppb)	0.001	0.001	0.229	0.001	0.001	0.189
Peripheral eosinophils (/μL)	0.000	0.000	0.960	−0.000	0.000	0.930
Peripheral neutrophils (/μL)	−0.000	0.000	0.946	0.000	0.000	0.916
Serum IgE (IU/mL)	−0.000	0.000	0.547	−0.000	0.000	0.702
Serum periostin (ng/mL)	−0.001	0.001	0.116	−0.002	0.001	0.036*
NK cells (% of lymphocytes)	0.005	0.004	0.249	0.004	0.004	0.314
ILC1 (% of Lin$^-$ CD127$^+$ CD161$^+$ cells)	−0.002	0.002	0.270	−0.002	0.002	0.284
ILC2 (% of Lin$^-$ CD127$^+$ CD161$^+$ cells)	0.002	0.002	0.251	0.003	0.002	0.237
ILC3 (% of Lin$^-$ CD127$^+$ CD161$^+$ cells)	−0.000	0.005	0.952	−0.001	0.005	0.840
CD69$^+$ NK cells (% of NK cells)	0.017	0.004	<0.001*	0.018	0.004	<0.001*
CD69$^+$ ILC1 (% of ILC1)	0.014	0.004	0.001*	0.015	0.004	<0.001*
CD69$^+$ ILC2 (% of ILC2)	0.006	0.003	0.072	0.007	0.003	0.041*
CD69$^+$ ILC3 (% of ILC3)	0.004	0.002	0.041*	0.004	0.002	0.034*

*Indicates $p < 0.05$.

and have been shown to produce Th2 cytokines such as IL-5 and IL-13 in the development of allergic asthma.[39] Epithelial cell-derived cytokines such as IL-33 was correlated with the Th2 cytokines and provoked IL-13-producing ILC2s, which have a preeminent role in asthma pathogenesis.[29,40,41] Halim *et al.* have shown that ILC2s regulated the differentiation of naive $CD4^+$ T cells into Th2 cells.[42] Moreover, it has been reported that IL-17- and IL-22-producing ILC3s facilitate obesity-associated asthma.[43,44] The correlation between BMI and the circulating activated ILC3s in this study may reflect these reports that ILC3s mediates obesity-associated asthma. The MAIT cells also can produce IL-17, which is a potent activator of neutrophils, in human obesity and in murine models of arthritis.[10,45] Taken together, the positive correlation between activated NK cells, ILC1s, ILC2s, ILC3s, and MAIT cells in asthma suggested that antigen-specific Th2 cells and these innate subsets of lymphoid cells may have a role in orchestrating asthmatic airway inflammation.

Several studies have shown that ILC2s in human peripheral blood were significantly increased in patients with asthma.[46–48] Smith *et al.* have shown that absolute numbers of ILC2s, which were identified as Lin^- $CD45^+$ $CD127^+$ $ST2^+$ cells, were significantly increased in severe asthmatics, compared to mild asthmatics.[46] Bartemes *et al.* also have demonstrated the total numbers of ILC2s, which were identified Lin^- $CD127^+$ $CRTH2^+$ cells, were significantly increased in allergic asthmatics, compared to subjects with allergic rhinitis and healthy control.[47] Moreover, Liu *et al.* have reported the frequency of ILC2s identified by the ratio of Lin^- $CD127^+$ $CRTH2^+$ cells to peripheral blood lymphocytes were higher in eosinophilic asthmatics, compared to noneosinophilic asthmatics and healthy controls.[48] However, Barnig *et al.* have demonstrated that the frequency of ILC2s identified by the ratio of Lin^- $CD127^+$ $CRTH2^+$ cells to Lin^- $CD127^+$ cells in peripheral blood was not different between healthy individuals, mild asthmatics, and severe asthmatics.[28] In the present study, there were no significant differences between the asthma severity groups in the frequency of ILC2s identified by the ratio of Lin^- $CD127^+$ $CD161^+$ $CRTH2^+$ cells to Lin^- $CD127^+$ $CD161^+$ cells in peripheral blood. The frequency of ILC2s identified by the ratio may lead to these discrepant findings of asthmatics, because ratio can be influenced by the frequency of other cell subsets and the subsets of ILC2s were only a very small fraction.[29] Barnig *et al.* also have demonstrated $CD69^+$ Lin^- $CD56^+$ NK cells were increased in severe asthmatics, compared to healthy individuals and mild asthmatics.[28] Moreover, the frequency of MAIT cells in the peripheral blood was decreased in patients with severe asthma, multiple sclerosis, and inflammatory bowel disease.[9,24,36,49,50] Hinks *et al.* showed that decreased MAIT cell frequencies in the peripheral blood and lung tissue were correlated with severe asthma.[9] By contrast, in the current study, the peripheral blood NK cells, ILCs, and MAIT cells were not correlated with asthma severity. It is acknowledged that a limitation of current study was a lack of a healthy control group. Furthermore, the baseline characteristics of eligible patients in this study showed that the severe asthmatic group had lower FeNO levels and higher peripheral neutrophil counts. These findings suggest dominant neutrophilic airway inflammation. In addition, FEV_1% was higher in severe asthmatics than in mild to moderate asthmatics. The reason for the lack of correlation between the innate subsets of lymphoid cells and asthma severity and the high FEV_1% in severe asthmatics was not clear in the current study; however, it may be related to their asthma treatment (e.g., oral and inhaled corticosteroids) and to dominant neutrophilic airway inflammation in patients with severe asthma. In any event, this unique population subject may lead to discrepant findings between previous studies and the current study. Further studies are needed to investigate the association between the innate subsets of lymphoid cells and asthma severity.

In conclusion, to our knowledge, we have provided the first report that the $CD69^+$-activated NK cells, ILC1s, ILC2s, ILC3s, and MAIT cells were positively correlated with each other, and high activation of these cells may be associated with airflow limitation in patients with asthma. All of these activated populations may orchestrate immune responses, but not behave as an individual population, in asthmatic airway inflammation. However, these conclusions are limited by our small sample size. The innate subsets of lymphoid cells in allergic asthma require further investigation.

Acknowledgements

This work was supported in part by JSPS KAKENHI Grant Number 25461167.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.alit.2016.07.005

Conflict of interest

JO is an employee of Shino-Test Corporation. The rest of the authors have no conflict of interest.

Authors' contributions

AI, NH, AC, KT, and SM participated in the design of the study and drafted the manuscript. AI, NH, SH, KM, FM, JI, SO, JO, RA and KI contributed to data collection. AI, NH, SH, and SM performed the statistical analysis and interpretation of the results. All authors have read and approved the final manuscript.

References

1. Busse WW, Lemanske Jr RF. Asthma. *N Engl J Med* 2001;**344**:350–62.
2. Wills-Karp M. Immunologic basis of antigen-induced airway hyperresponsiveness. *Annu Rev Immunol* 1999;**17**:255–81.
3. Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;**326**:298–304.
4. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med* 2012;**18**:716–25.
5. Hogan SP, Mould A, Kikutani H, Ramsay AJ, Foster PS. Aeroallergen-induced eosinophilic inflammation, lung damage, and airways hyperreactivity in mice can occur independently of IL-4 and allergen-specific immunoglobulins. *J Clin Invest* 1997;**99**:1329–39.
6. Foster PS, Hogan SP, Ramsay AJ, Matthaei KI, Young IG. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J Exp Med* 1996;**183**:195–201.
7. Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. *Science* 1998;**282**:2258–61.
8. Gapin L. Where do MAIT cells fit in the family of unconventional T cells? *PLoS Biol* 2009;**7**:e70.
9. Hinks TS, Zhou X, Staples KJ, Dimitrov BD, Manta A, Petrossian T, et al. Innate and adaptive T cells in asthmatic patients: relationship to severity and disease mechanisms. *J Allergy Clin Immunol* 2015;**136**:323–33.
10. Chiba A, Tajima R, Tomi C, Miyazaki Y, Yamamura T, Miyake S. Mucosal-associated invariant T cells promote inflammation and exacerbate disease in murine models of arthritis. *Arthritis Rheum* 2012;**64**:153–61.
11. Croxford JL, Miyake S, Huang YY, Shimamura M, Yamamura T. Invariant V(alpha)19i T cells regulate autoimmune inflammation. *Nat Immunol* 2006;**7**:987–94.
12. Howson LJ, Salio M, Cerundolo V. MR1-restricted mucosal-associated invariant T cells and their activation during infectious diseases. *Front Immunol* 2015;**6**:303.
13. Spits H, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, et al. Innate lymphoid cells – a proposal for uniform nomenclature. *Nat Rev Immunol* 2013;**13**:145–9.
14. Hazenberg MD, Spits H. Human innate lymphoid cells. *Blood* 2014;**124**:700–9.
15. Spits H, Di Santo JP. The expanding family of innate lymphoid cells: regulators and effectors of immunity and tissue remodeling. *Nat Immunol* 2011;**12**:21–7.
16. Saenz SA, Noti M, Artis D. Innate immune cell populations function as initiators and effectors in Th2 cytokine responses. *Trends Immunol* 2010;**31**:407–13.
17. Napier RJ, Adams EJ, Gold MC, Lewinsohn DM. The role of mucosal associated invariant T cells in antimicrobial immunity. *Front Immunol* 2015;**6**:344.
18. Edwards SC, McGinley AM, McGuinness NC, Mills KH. Gammadelta T cells and NK cells – distinct pathogenic roles as innate-like immune cells in CNS autoimmunity. *Front Immunol* 2015;**6**:455.

19. Martin E, Treiner E, Duban L, Guerri L, Laude H, Toly C, et al. Stepwise development of MAIT cells in mouse and human. *PLoS Biol* 2009;**7**:e54.
20. Dusseaux M, Martin E, Serriari N, Peguillet I, Premel V, Louis D, et al. Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161hi IL-17-secreting T cells. *Blood* 2011;**117**:1250–9.
21. Kjer-Nielsen L, Patel O, Corbett AJ, Le Nours J, Meehan B, Liu L, et al. MR1 presents microbial vitamin B metabolites to MAIT cells. *Nature* 2012;**491**:717–23.
22. Jo J, Tan AT, Ussher JE, Sandalova E, Tang XZ, Tan-Garcia A, et al. Toll-like receptor 8 agonist and bacteria trigger potent activation of innate immune cells in human liver. *PLoS Pathog* 2014;**10**:e1004210.
23. Miyazaki Y, Miyake S, Chiba A, Lantz O, Yamamura T. Mucosal-associated invariant T cells regulate Th1 response in multiple sclerosis. *Int Immunol* 2011;**23**:529–35.
24. Willing A, Leach OA, Ufer F, Attfield KE, Steinbach K, Kursawe N, et al. CD8(+) MAIT cells infiltrate into the CNS and alterations in their blood frequencies correlate with IL-18 serum levels in multiple sclerosis. *Eur J Immunol* 2014;**44**:3119–28.
25. Nausch N, Appleby LJ, Sparks AM, Midzi N, Mduluza T, Mutapi F. Group 2 innate lymphoid cell proportions are diminished in young helminth infected children and restored by curative anti-helminthic treatment. *PLoS Negl Trop Dis* 2015;**9**:e0003627.
26. Mjosberg JM, Trifari S, Crellin NK, Peters CP, van Drunen CM, Piet B, et al. Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CRTH2 and CD161. *Nat Immunol* 2011;**12**:1055–62.
27. Kim BS, Siracusa MC, Saenz SA, Noti M, Monticelli LA, Sonnenberg GF, et al. TSLP elicits IL-33-independent innate lymphoid cell responses to promote skin inflammation. *Sci Transl Med* 2013;**5**:170ra16.
28. Barnig C, Cernadas M, Dutile S, Liu X, Perrella MA, Kazani S, et al. Lipoxin A4 regulates natural killer cell and type 2 innate lymphoid cell activation in asthma. *Sci Transl Med* 2013;**5**:174ra26.
29. Kabata H, Moro K, Koyasu S, Asano K. Group 2 innate lymphoid cells and asthma. *Allergol Int* 2015;**64**:227–34.
30. Global Initiative for Asthma (GINA). Global Strategy for Asthma Management and Prevention. Updated 2014. Available from: http://www.ginasthma.org/.
31. Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global Strategy for Diagnosis, Management, and Prevention of COPD. Updated 2006. Available from: http://www.goldcopd.org/.
32. Okamoto M, Hoshino T, Kitasato Y, Sakazaki Y, Kawayama T, Fujimoto K, et al. Periostin, a matrix protein, is a novel biomarker for idiopathic interstitial pneumonias. *Eur Respir J* 2011;**37**:1119–27.
33. Munneke JM, Bjorklund AT, Mjosberg JM, Garming-Legert K, Bernink JH, Blom B, et al. Activated innate lymphoid cells are associated with a reduced susceptibility to graft-versus-host disease. *Blood* 2014;**124**:812–21.
34. Dalbeth N, Gundle R, Davies RJ, Lee YC, McMichael AJ, Callan MF. CD56bright NK cells are enriched at inflammatory sites and can engage with monocytes in a reciprocal program of activation. *J Immunol* 2004;**173**:6418–26.
35. Cooper MA, Fehniger TA, Turner SC, Chen KS, Ghaheri BA, Ghayur T, et al. Human natural killer cells: a unique innate immunoregulatory role for the CD56(bright) subset. *Blood* 2001;**97**:3146–51.
36. Hiejima E, Kawai T, Nakase H, Tsuruyama T, Morimoto T, Yasumi T, et al. Reduced numbers and proapoptotic features of mucosal-associated invariant T cells as a characteristic finding in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2015;**21**:1529–40.
37. Novak J, Dobrovolny J, Novakova L, Kozak T. The decrease in number and change in phenotype of mucosal-associated invariant T cells in the elderly and differences in men and women of reproductive age. *Scand J Immunol* 2014;**80**:271–5.
38. Holt PG, Sly PD. Viral infections and atopy in asthma pathogenesis: new rationales for asthma prevention and treatment. *Nat Med* 2012;**18**:726–35.
39. Mathias CB. Natural killer cells in the development of asthma. *Curr Allergy Asthma Rep* 2015;**15**:500.
40. Jackson DJ, Makrinioti H, Rana BM, Shamji BW, Trujillo-Torralbo MB, Footitt J, et al. IL-33-dependent type 2 inflammation during rhinovirus-induced asthma exacerbations in vivo. *Am J Respir Crit Care Med* 2014;**190**:1373–82.
41. Barlow JL, Peel S, Fox J, Panova V, Hardman CS, Camelo A, et al. IL-33 is more potent than IL-25 in provoking IL-13-producing nuocytes (type 2 innate lymphoid cells) and airway contraction. *J Allergy Clin Immunol* 2013;**132**:933–41.
42. Halim TY, Steer CA, Matha L, Gold MJ, Martinez-Gonzalez I, McNagny KM, et al. Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. *Immunity* 2014;**40**:425–35.
43. Kim HY, Lee HJ, Chang YJ, Pichavant M, Shore SA, Fitzgerald KA, et al. Interleukin-17-producing innate lymphoid cells and the NLRP3 inflammasome facilitate obesity-associated airway hyperreactivity. *Nat Med* 2014;**20**:54–61.
44. Taube C, Tertilt C, Gyulveszi G, Dehzad N, Kreymborg K, Schneeweiss K, et al. IL-22 is produced by innate lymphoid cells and limits inflammation in allergic airway disease. *PLoS One* 2011;**6**:e21799.
45. Carolan E, Tobin LM, Mangan BA, Corrigan M, Gaoatswe G, Byrne G, et al. Altered distribution and increased IL-17 production by mucosal-associated invariant T cells in adult and childhood obesity. *J Immunol* 2015;**194**:5775–80.
46. Smith SG, Chen R, Kjarsgaard M, Huang C, Oliveria JP, O'Byrne PM, et al. Increased numbers of activated group 2 innate lymphoid cells in the airways of patients with severe asthma and persistent airway eosinophilia. *J Allergy Clin Immunol* 2016;**137**:75–86.e8.
47. Bartemes KR, Kephart GM, Fox SJ, Kita H. Enhanced innate type 2 immune response in peripheral blood from patients with asthma. *J Allergy Clin Immunol* 2014;**134**: 671–8.e4.
48. Liu T, Wu J, Zhao J, Wang J, Zhang Y, Liu L, et al. Type 2 innate lymphoid cells: a novel biomarker of eosinophilic airway inflammation in patients with mild to moderate asthma. *Respir Med* 2015;**109**:1391–6.
49. Haga K, Chiba A, Shibuya T, Osada T, Ishikawa D, Kodani T, et al. MAIT cells are activated and accumulated in the inflamed mucosa of ulcerative colitis. *J Gastroenterol Hepatol* 2015;**31**:965–72.
50. Serriari NE, Eoche M, Lamotte L, Lion J, Fumery M, Marcelo P, et al. Innate mucosal-associated invariant T (MAIT) cells are activated in inflammatory bowel diseases. *Clin Exp Immunol* 2014;**176**:266–74.