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

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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.

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Peer review under responsibility of Japanese Society of Allergology.
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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