T follicular helper cells in peripheral blood are associated with the expression of B cells and plasma IgE level in patients with acute asthma exacerbation

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Abstract: Differentiation of T follicular helper (Tfh) cells was enhanced in patients with acute asthma exacerbation, but their associations with the expression of B cells and IgE level were not clear. In this study, we aimed to explore the correlations between Tfh cells and B cells, as well as plasma IgE, IgG1 levels. Peripheral blood samples were collected from forty-two patients with acute exacerbation and forty-eight matched controls. Tfh cells (CD4+CXCR5+) and B cells (CD19+) were examined by flow cytometry, while plasma IgE, IgG1 and IL-21 level were measured by enzyme-linked immunosorbent assay (ELISA). The percentage of Tfh cells (P<0.001), B cells (P<0.001) in peripheral blood, plasma IgE (P<0.001), IgG1 (P<0.001) and IL-21 (P<0.001) levels were all increased in patients with acute asthma exacerbation compared with health controls. The percentage of Tfh cells was associated with B cells (r=0.514, P<0.001) and plasma IgE level (r=0.620, P<0.001), while no significant correlation between the percentage of Tfh Cells and IgG1 (r=0.255, P=0.103) in asthma patients with acute exacerbation was observed. Similarly, plasma IL-21 level was associated with B cells (r=0.831, P<0.001) plasma IgE level (r=0.324, P=0.036), with no significant correlation between the plasma level of IL-21 and IgG1 (r=0.207, P=0.188) in asthma patients with acute exacerbation. Tfh cells may participate in the airway inflammation reaction through promoting the differentiation and IgE class switching of B cells in asthma patients with acute exacerbation.

Keywords: T follicular helper cells, B cells, plasma IgE, acute asthma exacerbation

Introduction

T follicular helper cells (Tfh cells) are the major cells which could induce the differentiation, proliferation and activation of B cells and help the class switching of the immunoglobulin antibody [1, 2]. It has been proved that both the differentiation and secretion function of Tfh cells would be enhanced in acute bronchial asthma (Hereinafter referred to as “asthma”) exacerbation [3, 4], but it still remained unclear whether it mediated the differentiation and secretion function of B cells in asthma. This study aimed to evaluate the differences of the molecular mark on the surface of Tfh cells (CD4+CXCR5+), the level of its primary secretion cytokines (IL-21), the molecular mark on the surface of B cells (CD19+) as well as plasma IgE and IgG1 between patients with acute asthma exacerbation and healthy volunteers, and to analyze the correlations of Tfh cells, IL-21 level with B cells, plasma IgE and IgG1 levels in peripheral blood, to investigate whether the enhancement of Tfh cells differentiation participated in the differentiation and secretion of B cells, which could lay the foundation for further study in the mechanism of Tfh cells in asthma.

Methods

Participants

42 patients with asthma from Department of Respiratory Medicine in the Central Hospital of Wuhan between June 2013 and May 2015 were included. Inclusion criteria: matched the classification of asthma of the American Thoracic Society criteria [5], patients with clinical symptoms of typical acute asthma exacerbation...
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<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patients with acute asthma exacerbation (n=42)</th>
<th>Controls (n=48)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>41±10</td>
<td>44±11</td>
<td>0.319</td>
</tr>
<tr>
<td>Female (%)</td>
<td>22 (52%)</td>
<td>23 (48%)</td>
<td>0.673</td>
</tr>
<tr>
<td>FVC (L)</td>
<td>2.1±0.3</td>
<td>3.2±0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt; (L)</td>
<td>1.3±0.2</td>
<td>2.6±0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;/FVC (ratio %)</td>
<td>60.7±2.9</td>
<td>82.5±3.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as Mean values ± SD or count and percentages. Significance of the comparison is determined by the Student t test and the χ² test. FVC, forced vital capacity; FEV<sub>1</sub>, forced expiratory volume in one second.

48 healthy volunteers from physical examination department of the Central Hospital of Wuhan were included as controls. Inclusion criteria: no history of asthma, non-allergic constitution, negative results in allergy skin test, normal lung function, no history of respiratory disease, no history of immune or tumor diseases, no acute infection within 4 weeks.

All the participants agreed to participate in this study and signed the written informed consent. This clinical protocol has been approved by the Ethics committee of the Central Hospital of Wuhan.

Blood sample

The peripheral blood samples from patients with acute asthma exacerbation were collected before the treatment, the peripheral blood samples from healthy volunteers in control group were collected during the physical examination.

Identification of Tfh cells and B cells levels in peripheral blood by flow cytometry

5–8 ml venous blood from every subject was collected by heparin sodium anticoagulation pipets, and centrifuged for 8 min in 800 r/min at room temperature, the supernatant was taken and cryopreserved in -80°C fridge. Then it was diluted by PBS, the peripheral blood mononuclear cells (PBMCs) were separated according to the instruction of Ficoll Lymphocyte separation medium (Nycomed company, Norway), the washed cells were collected, divided into two groups and added with anti-human CD4-PE, CXCR5-FITC, CD19-PE antibody (all from eBioscience company, USA), the mice IgG marked by FITC and the rabbit IgG<sub>2a</sub> marked by PE were Isotype controls. Incubated for 40 min away from light, washed twice by PBS, after fixed with fixative (eBioscience company, USA), tested the expression proportion of the molecular mark on the surface of the cells by BD FACS AriaTM flow cytometry. Expression proportion of CD4<sup>+</sup>-CXCR5<sup>+</sup> cells (Tfh cells) and CD19<sup>+</sup> cells (B cells) was determined by lymphoid cells in PBMCs.

Identification of the plasma IL-21, IgE and IgG<sub>1</sub> levels in peripheral blood by Elisa

The peripheral blood plasma from the subjects was collected and cryopreserved, the IL-21, IgE and IgG<sub>1</sub> levels were examined by Elisa according to the method described in the instruction (all from eBioscience company, USA). The detection range of IL-21 was 8–1000 pg/mL, the detection range of IgE was 7.8–1000 ng/mL, the detection range of IgG<sub>1</sub> was 0.16–10 μg/ml. All the samples were tested for three times repeatedly.

Statistical analysis

Data was presented as mean values ± SD, median and 25th-75th quartile or percentages. The variation analysis was conducted by Student t, Mann-Whitney or χ² test; Pearson and Spearman test were used for correlation analysis. All the statistical analysis was conducted using SAS 8.1, P<0.05 was defined as statistical significance.

Results

Characteristics of patients with acute asthma exacerbation and health controls

There was no significant difference between the age (41±10 years old) and gender (female...
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Table 2. Tfh cells (CD4+CXCR5+) and B cells (CD19+) in patients with acute asthma exacerbation and controls

<table>
<thead>
<tr>
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<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+CXCR5+</td>
<td>22.53±5.40%</td>
<td>17.21±4.52%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD19+</td>
<td>6.79±2.57%</td>
<td>3.05±1.69%</td>
<td>&lt;0.001</td>
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</table>

Data are presented as Mean values ± SD. Significance of the comparison is determined by the student t test.

Table 3. Plasma levels of IgE, IgG, IL-21 in patients with acute asthma exacerbation and controls

<table>
<thead>
<tr>
<th>Parameters</th>
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<th>Controls (n=48)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgE (ng/ml)</td>
<td>88.42 (39.40-172.93)</td>
<td>39.65 (18.54-68.72)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IgG (ug/ml)</td>
<td>0.908 (0.473-1.217)</td>
<td>0.542 (0.342-0.813)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-21 (pg/ml)</td>
<td>181.61 (68.21-221.67)</td>
<td>78.42 (33.49-136.11)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as median and 25th-75th quartile. Significance of the comparison is determined by the Mann-Whitney test.

22/52%) of the patients with acute asthma exacerbation and the health controls (age 44±11 years old, female 23/48%), P=0.319 and P=0.673, respectively. As shown in Table 1.

Tfh and B cells ratio, plasma IL-21, IgE and IgG levels in patients with acute asthma exacerbation

CD4+CXCR5+ cells (Tfh cells) proportion and CD19+ cells (B cells) proportion in the lymphocyte of patients with acute asthma exacerbation were significantly higher than that in health controls (22.53±5.40% vs. 17.21±4.52%, P<0.001; 6.79±2.57% vs. 3.05±1.69%, P<0.001), presented in Table 2. Besides, plasma levels of IL-21, IgE, IgG were remarkably elevated in asthma exacerbation patients compared with health controls [181.61 (68.21-221.67) vs. 78.42 (33.49-136.11), P<0.001; 88.42 (39.40-172.93) vs. 39.65 (18.54-68.72), P<0.001; 0.908 (0.473-1.217) vs. 0.542 (0.342-0.813), P<0.001, respectively], as shown in Table 3.

Correlations analysis of Tfh ratio with B cells ratio, IgE and IgG levels in patients with acute asthma exacerbation

CD4+CXCR5+ cells (Tfh cells) proportion and CD19+ cells (B cells) proportion were positive correlated in patients with acute asthma exacerbation (r=0.514, P<0.001) (Figure 1A). Similarly, the Tfh cells proportion was positively correlated to the plasma IgE level in peripheral blood (r=0.620, P<0.001) (Figure 1B). However, Tfh cells proportion and plasma IgG level in peripheral blood did not present any significant correlation (r=0.103) (Figure 1C).

Correlation analysis of the IL-21 level and B cells level, IgE, IgG, in peripheral blood plasma of patients with acute asthma exacerbation

Plasma IL-21 level in peripheral blood presented positive correlation with CD19+ (B cells) in lymphocyte (r=0.831, P<0.001) (Figure 2A) in patients with acute bronchial asthma exacerbation, as well as IgE level (r=0.324, P=0.036) (Figure 2B). However, plasma IL-21 and IgG level did not show any significant correlation (r=0.207, P=0.188) (Figure 2C).

Discussion

In the pathogenesis of asthma, both the cellular immunity mediated by T helper cells and the humoral immunity mediated by B cells participates in the disease process [6, 7]. The humoral immunity induced by IgE and IgG from B cells is the primary immunologic mechanism of asthma [8]. In the mice model of bronchial asthma, the formation of germinal center, activation of B cells and secretion of IgE could be observed in lymphoid organ and inflammatory lung tissue. Meanwhile, the activation, proliferation and differentiation of B cells are mediated by the T lymphocyte subpopulation: T Follicular helper (Tfh) cells [1, 2]. Currently, it has been demonstrated that the differentiation of Tfh cells is increased in the patients with acute asthma exacerbation [3, 4], but it is still not clear whether the enhancement of Tfh cells differentiation and secretion were associated with B cells.

IL-21 is the most important cytokine in secretion of Tfh cells, which contributes to the adaptive proliferation of B cells in germinal center [9,
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Both in vivo and in vitro test, IL-21 promotes the differentiation of the plasma cells via activating STAT3 [11-13], meanwhile increasing the IL-4 mediated transformation of IgE in

Figure 1. The correlation of Tfh cells with B cells, plasma IgE level and IgG level. A. Analyzed by Pearson test; B and C. Analyzed by Spearman test.

Figure 2. The correlation of plasma IL-21 level with B cells, plasma IgE level and IgG level. Analyzed by spearman test.
human B cells through the STAT3-dependent pathway [14]. In addition, IL-21 increases the Tfh cells proportion in vivo and vitro conversely, indicates the interaction between IL-21 and Tfh cells [15].

In this study, we presented that the proportion of Tfh cells in patients with acute asthma exacerbation and IL-21 level in peripheral blood plasma were positively correlated to B cells, which indicated that the differentiation of Tfh cells and increased secretion of IL-21 might contribute to the proliferation and differentiation of B cells during the onset of asthma. In patients with eosinophils nasal polyps, the number of Tfh cells presents positive correlation with the maturity of B cells in germinal center [16]. While the proportion of Tfh cells and IL-21 level in peripheral blood plasma in patients with acute asthma exacerbation were positively correlated to IgE level in peripheral blood plasma, which indicated that the differentiation of Tfh cells stimulated the class switching of IgE in B cells. In eosinophils nasal polyps, the investigators found that the proportion of IL-21+ Tfh cells are positively related to IgE level in plasma [16].

IgG1 level was increased in asthma alveolar lavage fluid [17, 18]. While in plasma, in our previous study, we have demonstrated that the IgG1 level of patients with acute asthma exacerbation was apparently higher than that in health control group, and increased IgG1 level was positively correlated to the enhancement of differentiation of B cells [19]. However, in present study, T cells in patients with acute asthma exacerbation and the IL-21 level in peripheral blood plasma showed no obvious correlation with the secretion of IgG1 in peripheral blood plasma, which indicated that Tfh cells might not participate in the class switching of IgG1. Early study found that the IgG1 level is evaluated in children who were allergic to house dust compared with children without allergy, but the IgG1 level in the acute exacerbation was lower than that in the remission status [20]. Another study on patients who were allergic to birch pollen also found that IgG1 suppresses the anaphylaxis reaction. They believed that IgG1 probably inhibits the combination between IgE and its target site through competing combination of itself, to suppress the allergy reaction [21]. So currently it is still controversial what role of IgG and its subtype in Th2 airway allergic reaction are, and it needs further study on whether there is any other mechanism in the finding showed in present study that there was no correlation between the differentiation of Tfh cells and IgG1.

This study presented that Tfh cells was positively correlated to B cells and plasma IgE level in patients with acute asthma exacerbation through correlation analysis, as well as IL-21, which indicated that Tfh cells and IL-21 might promote the participation of class switching of IgE in airway inflammation in asthma via stimulating the proliferation of B cells. Next step, we will investigate in animal experiment whether the direct blocking of the differentiation of Tfh cells could affect the differentiation and function of B cells in asthma mice model.

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Disclosure of conflict of interest

None.

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References


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[5] Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. This official statement of the American thoracic society was adopted by the ATS board of directors, November 1986. Am Rev Respir Dis 1987; 136: 225-44.


