The relationship of different respiratory virus infection with pediatric asthma attack as well as cytokine and lymphocyte subset levels

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ARTICLE INFO

Article history:
Received 13 Apr 2017
Received in revised form 16 Apr 2017
Accepted 19 Apr 2017
Available online 24 May 2017

Keywords:
Bronchial asthma
Respiratory viruses
Airway function
CD4+ T lymphocyte

ABSTRACT

Objective: To study the relationship of different respiratory virus infection with pediatric asthma attack as well as cytokine and lymphocyte subset levels. Methods: A total of 85 children who were diagnosed with bronchial asthma in our hospital between May 2013 and March 2016 were selected as asthma group and further divided into asthma-RSV group, asthma-AV group, asthma-PIV group, asthma-IFV group and pure asthma group according to the condition of respiratory virus infection, and 70 healthy children who received physical examination in our hospital during the same period were selected as the control group. Spirometer was used to determine airway function parameters, enzyme-linked immunosorbent assay kits were used to determine serum cytokine contents, and flow cytometry was used to determine peripheral blood lymphocyte subset contents. Results: FEV1/FVC, FEF25, FEF50 and FEF75 levels, serum IL-2, IFN-γ and TGF-β1 contents as well as peripheral blood Th1 and Treg cell contents of asthma groups were significantly lower than those of control group while serum IL-4, IL-5 and IL-17 contents as well as peripheral blood Th2 and Th17 cell contents were significantly higher than those of control group; FEV1/FVC, FEF25, FEF50 and FEF75 levels, serum IL-2, IFN-γ and TGF-β1 contents as well as peripheral blood Th1 and Treg cell contents of asthma-RSV group and asthma-IFV group were significantly lower than those of pure asthma group while serum IL-4, IL-5 and IL-17 contents as well as peripheral blood Th2 and Th17 cell contents were significantly higher than those of pure asthoma group; these indexes of asthma-AV group and asthma-PIV group were not significantly different from those of pure asthma group. Conclusion: RSV and IFV infection can affect the airway function and the balance of CD4+ T cell subsets to promote the development of asthma.

1. Introduction

Bronchial asthma is a common disease of pediatric respiratory system, its basic pathological characteristics are airway hyperresponsiveness, reversible spasms and chronic inflammation, and the lymphocyte dysfunction and cytokine secretion disorder are the important factors causing the occurrence and development of bronchial asthma[1-3]. However, it is not clear at present about the specific mechanism of lymphocyte function change in children with bronchial asthma. Respiratory virus infection is one of the common causes of acute asthma attacks[4,5], virus infection can cause the change in immune response to the secretion of corresponding cytokines in the body, and study also shows that respiratory virus infection can aggravate the disease severity in animal models with asthma[6]. Respiratory syncytial virus (RSV), adenovirus (AV), parainfluenza virus (PIV) and influenza virus (IFV) are the common pathogens that cause infantile respiratory tract infection, and in order to define the relationship of respiratory virus infection with bronchial asthma occurrence and attack, the correlation of different respiratory virus infection with pediatric asthma attack as well as cytokine and lymphocyte subset levels was analyzed in the following study.
2. Subjects and methods

2.1 Research subjects

A total of 85 children who were diagnosed with bronchial asthma and 70 healthy children who received physical examination in our hospital between May 2013 and March 2016 were selected as the research subjects. Children with bronchial asthma were clearly diagnosed by airway function examination and the children who took glucocorticoid and β2 agonists within the last one month were excluded; healthy children were proven healthy after physical examination, and without the history of respiratory tract infection within the last three months.

2.2 Research groups

Healthy children were selected as control group, children with asthma were selected as asthma group, and the children with asthma were further divided into asthma-RSV group, asthma-AV group, asthma-PIV group, asthma-IFV group and pure asthma group according to the condition of respiratory virus infection.

2.3 Index detection methods

(1) Airway function detection: airway function detection parameters FEV1, FVC, FEF25, FEF50, FEF75 of two groups of children were measured by pulmonary function meter, then FEV1/FVC was calculated. (2) serum cytokines detection: 3ml of cubital venous blood was collected from the control group and asthma group and centrifuged to separate serum, and then enzyme-linked immunosorbent assay kits were used to determine IL-2, IL-4, IL-5, IL-17, IFN-γ and TGF-β1 contents. (3) Peripheral blood T cell subset detection: 3 mL of cubital venous blood was collected from the control group and asthma group, anti-coagulated with EDTA to incubate fluorescent antibodies of CD3, CD4, CD25, Foxp3, IL-4, IL-17 and IFN-γ for 20 min, then added in red blood cell lysis buffer for continuous incubation for 15 min, finally washed with PBS and centrifuged twice to determine Th1, Th2, Th17 and Treg cell contents by flow cytometer.

2.4 Statistical processing

SPSS 17.0 software was used to input the data for airway function parameters, serum cytokines and peripheral blood lymphocyte subsets, comparison of above data among groups was by variance analysis and the data with differences after variance analysis was further by pair-wise comparison with LSD-t test. P<0.05 indicated statistical significance in differences.

3. Results

3.1 Airway function parameters

FEV1/FVC, FEF25 (L/s), FEF50 (L/s) and FEF75 (L/s) of asthma-RSV group, asthma-AV group, asthma-PIV group, asthma-IFV group and pure asthma group were significantly lower than those of control group, FEV1/FVC, FEF25 and FEF75 of asthma-RSV group and asthma-IFV group were significantly lower than those of asthma-AV group, asthma-PIV group and pure asthma group, and FEV1/FVC, FEF25 and FEF75 of asthma-AV group and asthma-PIV group were not significantly different from those of pure asthma group, shown in Table 1.

3.2 Serum cytokine contents

Serum IL-2 (pg/mL), IFN-γ (ng/mL) and TGF-β1 (ng/mL) contents of asthma-RSV group, asthma-AV group, asthma-PIV group, asthma-IFV group and pure asthma group were significantly lower than those of control group while serum IL-4 (ng/mL), IL-5 (ng/mL) and IL-17 (ng/mL) contents were significantly higher than those of pure asthma group, serum IL-2, IFN-γ and TGF-β1 contents of asthma-RSV group and asthma-IFV group were significantly lower than those of pure asthma group while serum IL-4, IL-5 and IL-17 contents were significantly higher than those of pure asthma group and asthma-PIV group were not significantly different from those of pure asthma group, shown in Table 1.

Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>FEV1/FVC</th>
<th>FEF25</th>
<th>FEF50</th>
<th>FEF75</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthma-RSV group</td>
<td>17</td>
<td>0.76±0.10</td>
<td>0.70±0.09</td>
<td>1.09±0.14</td>
<td>1.79±0.23</td>
</tr>
<tr>
<td>Asthma-AV group</td>
<td>12</td>
<td>0.84±0.11</td>
<td>0.85±0.13</td>
<td>1.31±0.17</td>
<td>1.98±0.24</td>
</tr>
<tr>
<td>Asthma-PIV group</td>
<td>13</td>
<td>0.85±0.12</td>
<td>0.83±0.11</td>
<td>1.28±0.15</td>
<td>2.02±0.27</td>
</tr>
<tr>
<td>Asthma-IFV group</td>
<td>15</td>
<td>0.79±0.09</td>
<td>0.73±0.07</td>
<td>1.06±0.12</td>
<td>1.73±0.21</td>
</tr>
<tr>
<td>Pure asthma group</td>
<td>29</td>
<td>0.86±0.10</td>
<td>0.86±0.12</td>
<td>1.35±0.18</td>
<td>2.05±0.22</td>
</tr>
<tr>
<td>Control group</td>
<td>70</td>
<td>0.92±0.11</td>
<td>1.15±0.16</td>
<td>1.73±0.21</td>
<td>2.48±0.35</td>
</tr>
</tbody>
</table>

*: compared with control group, P<0.05; &: compared with pure asthma group, P<0.05.


Table 2.
Comparison of serum cytokine contents among all groups of subjects.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>IL-2</th>
<th>IL-4</th>
<th>IL-5</th>
<th>IL-17</th>
<th>IFN-γ</th>
<th>TGF-β 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthma-RSV group</td>
<td>17</td>
<td>16.21±2.23*</td>
<td>2.85±0.32*</td>
<td>5.89±7.77*</td>
<td>80.52±10.25*</td>
<td>0.16±0.22*</td>
<td>0.14±0.02*</td>
</tr>
<tr>
<td>Asthma-AV group</td>
<td>12</td>
<td>24.52±3.62*</td>
<td>1.46±0.19*</td>
<td>4.21±0.97*</td>
<td>57.64±7.81*</td>
<td>0.25±0.28*</td>
<td>0.21±0.03*</td>
</tr>
<tr>
<td>Asthma-PIV group</td>
<td>13</td>
<td>23.91±4.14*</td>
<td>1.42±0.39*</td>
<td>3.98±6.62*</td>
<td>59.33±7.12*</td>
<td>0.27±0.21*</td>
<td>0.29±0.02*</td>
</tr>
<tr>
<td>Asthma-IFV group</td>
<td>15</td>
<td>16.62±2.04*</td>
<td>2.81±0.24*</td>
<td>5.48±7.77*</td>
<td>78.79±9.25*</td>
<td>0.17±0.02*</td>
<td>0.13±0.04*</td>
</tr>
<tr>
<td>Pure asthma group</td>
<td>29</td>
<td>23.12±3.85*</td>
<td>1.54±0.21*</td>
<td>4.02±0.59*</td>
<td>61.28±7.64*</td>
<td>0.29±0.30*</td>
<td>0.22±0.04*</td>
</tr>
<tr>
<td>Control group</td>
<td>70</td>
<td>39.51±7.52</td>
<td>1.02±0.15</td>
<td>2.32±0.42</td>
<td>30.25±5.52</td>
<td>0.46±0.07</td>
<td>0.28±0.03</td>
</tr>
</tbody>
</table>

*: compared with control group, P<0.05; #: compared with pure asthma group, P<0.05.

Table 3.
Comparison of peripheral blood lymphocyte subset contents among all groups of subjects.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Th1</th>
<th>Th2</th>
<th>Th17</th>
<th>Treg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma-RSV group</td>
<td>17</td>
<td>6.02±0.76*</td>
<td>8.57±0.93*</td>
<td>4.89±0.72*</td>
<td>2.61±0.35*</td>
</tr>
<tr>
<td>Asthma-AV group</td>
<td>12</td>
<td>7.24±0.94*</td>
<td>7.12±0.88*</td>
<td>3.72±0.52*</td>
<td>3.36±0.47*</td>
</tr>
<tr>
<td>Asthma-PIV group</td>
<td>13</td>
<td>7.61±0.89*</td>
<td>6.97±0.92*</td>
<td>3.80±0.57*</td>
<td>3.42±0.49*</td>
</tr>
<tr>
<td>Asthma-IFV group</td>
<td>15</td>
<td>6.15±0.79*</td>
<td>8.61±1.02*</td>
<td>4.95±0.68*</td>
<td>2.69±0.37*</td>
</tr>
<tr>
<td>Pure asthma group</td>
<td>29</td>
<td>6.34±0.93*</td>
<td>6.82±0.89*</td>
<td>3.69±0.44*</td>
<td>3.65±0.41*</td>
</tr>
<tr>
<td>Control group</td>
<td>70</td>
<td>10.86±1.85</td>
<td>5.41±0.77</td>
<td>2.25±0.34</td>
<td>5.92±0.77</td>
</tr>
</tbody>
</table>

*: compared with control group, P<0.05; #: compared with pure asthma group, P<0.05.

3.3 Peripheral blood lymphocyte subset contents

Peripheral blood Th1 and Treg cell contents of asthma-RSV group, asthma-AV group, asthma-PIV group, asthma-IFV group and pure asthma group were significantly lower than those of control group, while Th2 and Th17 cell contents were significantly higher than those of control group; peripheral blood Th1 and Treg cell contents of asthma-RSV group and asthma-IFV group were significantly lower than those of pure asthma group while Th2 and Th17 cell contents were significantly higher than those of pure asthma group; peripheral blood Th1, Th2, Th17 and Treg cell contents of asthma-AV group and asthma-PIV group were not significantly different from those of pure asthma group, shown in Table 3.

4. Discussion

Respiratory virus infection is the important cause of pediatric acute asthma attack, but the influence of virus infection on the occurrence and development of asthma is not yet clear. RSV, AV, PIV and IFV are the viruses that cause infantile respiratory tract infection[7,8], and in the study, in order to define the relationship of different respiratory virus infection with the occurrence and development of pediatric asthma, the airway function parameters were analyzed at first between groups, and the results showed that FEV1/FVC, FEF25 (L/s), FEF50 (L/s) and FEF75 (L/s) of asthma groups were significantly lower than those of control group. This means that airway resistance increases significantly in children with asthma. Further analysis of the influence of different respiratory virus infection on airway function parameters showed that FEV1/FVC, FEF25, FE50 and FE75 of asthma-RSV group and asthma-IFV group were significantly lower than those of pure asthma group, and FEV1/FVC, FEF25, FE50 and FE75 of asthma-AV group and asthma-PIV group were not significantly different from those of pure asthma group. It means that respiratory RSV and IFV infection may affect the airway function in children with asthma, and AV and PIV infection will not significantly influence the airway function of children with asthma. In combination with the pathogenesis of bronchial asthma, it is further speculated that RSV and IFV infection may affect the content and function of lymphocyte subsets as well as the synthesis and secretion of cytokines to change the condition of bronchial asthma.

T lymphocyte is the important cell mass regulating cellular immune response, and the disorder of peripheral blood CD4+ helper T lymphocyte subset content and function is an important characteristic of children with bronchial asthma[9,10]. Th1 and Th2 are the first discovered CD4+T cell subsets, the former mainly secretes cytokines such as IL-2 and IFN-γ, and the latter mainly secretes cytokines such as IL-4 and IL-5[11]. Under physiological conditions, the functions of Th1 and Th2 influence each other and are in balance; in the pathological process of asthma, Th1 and Th2 balance is broken, which is specifically characterized by the weakened Th1 subset function and the enhanced Th2 subset function[12,13]. In the study, analysis of serum Th1 and Th2 cytokine contents as well as peripheral blood Th1 and Th2 cell contents in children with asthma...
confirmed that serum IL-2 and IFN-γ contents as well as peripheral blood Th1 cell contents of asthma groups were significantly lower than those of control group while serum IL-4 and IL-5 contents as well as peripheral blood Th2 cell contents were significantly higher than those of control group. Further analysis of the influence of different respiratory virus infection on Th1/Th2 balance showed that serum IL-2 and IFN-γ contents as well as peripheral blood Th1 cell contents of asthma-RSV group and asthma-IFV group were significantly lower than those of pure asthma group while serum IL-4 and IL-5 contents as well as peripheral blood Th2 cell contents were significantly higher than those of pure asthma group; these indexes of asthma-AV group and asthma-PIV group were not significantly different from those of pure asthma group. It means that RSV and IFV infection can cause the Th1/Th2 balance shifting to Th2, enhance the function of Th2 cells and inhibit the function of Th1 cells.

Th17 and Treg are the new CD4+ helper T lymphocyte subsets discovered in recent years, the former mainly secretes IL-17, the latter mainly secretes TGF-β1, and Th17 cell function is inhibited by Treg cells [14,15]. In the development and change of asthma, Th17 and Treg content and function change significantly, the number of Th17 increases and the function is enhanced, and the secreted IL-17 increases and can aggravate airway inflammation [16]; the number of Treg cells decrease and the function is weakened, the inhibiting effect on Th17 cells is weakened and the inflammatory response mediated by Th17 cells is enhanced [17,18]. In the study, analysis of serum Th17 and Treg cell contents as well as peripheral blood Th17 and Treg cell contents in children with asthma confirmed that serum IL-17 contents as well as peripheral blood Th17 cell contents of asthma groups were significantly higher than those of control group while serum TGF-β1 contents as well as peripheral blood Treg cell contents were significantly lower than those of control group. Further analysis of the influence of different respiratory virus infection on Th17/Treg balance showed that serum IL-17 contents as well as peripheral blood Th17 cell contents of asthma-RSV group and asthma-IFV group were significantly higher than those of pure asthma group; these indexes of asthma-AV group and asthma-PIV group were not significantly different from those of pure asthma group. It means that RSV and IFV can cause Th17/Treg balance shifting to Th2, enhance the function of Th2 cells and inhibit the function of Th1 cells.

Respiratory RSV and IFV infection can cause the development and change of bronchial asthma, which is specifically characterized by affecting airway function, causing CD4+ T cell subset imbalance, strengthening the function of Th2 and Th17 cells and suppressing the function of Th1 and Treg cells.

References


