Evaluation of serum chemokine levels, induced sputum adhesion molecule levels and peripheral blood immune cell contents of children with bronchial asthma

Zhi-Jun Chen*

Neonatology Department, Boai Hospital of Zhongshan City, Guangdong Province 528400, China

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ABSTRACT

Objective: To evaluate serum chemokine levels, induced sputum adhesion molecule levels and peripheral blood immune cell contents of children with bronchial asthma. Methods: 60 cases of bronchial asthma children were selected as observation group of the research; 60 cases of healthy volunteers were selected as control group. Then serum was collected to detect chemokine levels, induced sputum was collected to detect adhesion molecule levels and peripheral blood was collected to detect immune cell contents. Results: (1) chemokines: compared with serum index of control group, serum IL-6, IL-8, Eotaxin, MDC, MCP1 and RANTES contents of observation group were higher; (2) adhesion molecules: compared with induced sputum index of control group, induced sputum integrin α4β1, α6β1 and Mac-1 as well as its ligand VCAM-1, ICAM-1 and CD23 contents of observation group were higher; (3) immune molecules: compared with peripheral blood immune molecule contents of control group, peripheral blood CD4+CD25+CD127low Treg and NK cell contents of observation group were lower; Th9, Th17 and Thfh cell contents were higher. Conclusion: increased contents of serum chemokines, induced sputum integrin molecules and their ligands, and abnormal contents of peripheral blood immune molecules are related to the occurrence of bronchial asthma.

1. Introduction

Bronchial asthma is a common airway disease in children. Its pathological characteristics include nonspecific airway inflammation, increased airway responsiveness and airway remodeling, etc. Its pathogenesis mechanism is quite complex and is still not fully explained until now. It is a type of nonspecific inflammatory disease. Infiltration of inflammatory cells in the airway is closely related to its occurrence; chemokines and adhesion molecules are important molecules that mediate the recruitment and infiltration of inflammatory cells in local lesion, and content abnormality may cause the occurrence of the disease. Besides, immune dysfunction and abnormal levels of immune cells can be involved in the occurrence of bronchial asthma, too. To further clarify the mechanism that may be involved in the occurrence of bronchial asthma, serum chemokine levels, induced sputum adhesion molecule levels and peripheral blood immune cell contents of children with bronchial asthma were evaluated in the following research.

2. Objects and methods

2.1. Objects

Bronchial asthma children were selected by doctors of the same research group as observation group. They were diagnosed of bronchial asthma in our hospital outpatients from May 2012 to
September 2014, total 60 cases. All were at remission stage of bronchial asthma, including males/females: 38 cases/22 cases with age range of (8.92±0.82) and disease course of (5.28±0.68) months; healthy volunteers were selected as control group. They passed medical examination and didn’t have allergic disease or airway disease, including males/females: 41 cases/19 cases with age range of (8.87±0.86). There were no differences between the two groups’ general data ($P$>0.05).

2.2. Methods

2.2.1. Collecting methods of test specimens

Blood specimen was collected as follows: 4-6 mL of peripheral venous anti-clotting blood was collected at 7:00-7:30 in the morning and equally divided into two, one for FCM test and the other for Elisa test after centrifugation. Induced sputum was collected as follows: 4.5% atomized hypertonic saline was inhaled, mouth and nasal cavity were cleaned, and then repeated deep cough was conducted. Doctors of the same group determined the sputum volume, about 1.0-1.2 g sputum. Corresponding volume of DTT solution was added to it, then mixed and centrifuged. Supernatant was collected as induced sputum specimen.

2.2.2. Index detecting methods

Elisa kit was used to detect serum and induced sputum specimen IL-6, IL-8, Eotaxin, MDC, MCP1, and RANTES contents. After antibodies of marker molecules on immune cell surface were incubated, FCM was used to detect CD4$^+$/CD25$^+$/CD127$^{\text{low}}$ Treg, NK, Th9, Th17 and Tfh cell contents.

2.2.3. Statistical methods

Detected data was input by SPSS19.0 software, measurement data for $t$ test. Differences were considered to be significant at a level of $P$<0.05.

3. Results

3.1. Serum chemokines

Serum specimens of bronchial asthma children and healthy children were collected respectively. Then Elisa kit was used to detect chemokine molecule IL-6, IL-8, Eotaxin, MDC, MCP1 and RANTES contents. Statistical analysis showed that compared with serum index of control group, serum IL-6, IL-8, Eotaxin, MDC, MCP1 and RANTES contents of observation group were higher. Differences had statistical significance ($P$<0.05) (Table 1).

3.2. Induced sputum adhesion molecules

Induced sputum specimens of bronchial asthma children and healthy children were collected respectively. Then Elisa kit was used to detect integrin $\alpha_4\beta_1$, $\alpha_5\beta_6$ and Mac-1 as well as its ligand VCAM-1, ICAM-1 and CD23 contents. Statistical analysis showed that compared with induced sputum index of control group, induced sputum integrin $\alpha_4\beta_1$, $\alpha_5\beta_6$ and Mac-1 as well as its ligand VCAM-1, ICAM-1 and CD23 contents were higher. Differences had statistical significance ($P$<0.05) (Table 2).

3.3. Peripheral blood immune cells

Peripheral blood specimens of bronchial asthma children and healthy children were collected respectively. Then Elisa kit was used to detect chemokine molecule IL-6, IL-8, Eotaxin, MDC, MCP1 and RANTES contents. Statistical analysis showed that compared with serum index of control group, serum IL-6, IL-8, Eotaxin, MDC, MCP1 and RANTES contents of observation group were higher. Differences had statistical significance ($P$<0.05) (Table 1).

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healthy children were collected respectively. Antibodies of marker molecules on immune cell surface were incubated and then contents were detected. Statistical analysis showed that compared with peripheral blood immune molecule contents of control group, peripheral blood CD4\(^+\)CD25\(^-\)CD127\(^{low}\)Treg and NK cell contents of observation group were lower; Th9, Th17 and Tfh cell contents were higher. Differences had statistical significance (P<0.05) (Table 3).

4. Discussions

Bronchial asthma is a kind of chronic inflammatory airway disease. Infiltration of inflammatory cells in the airway is an important pathological feature of the disease. Chemokines are a type of cytokines that can recruit a variety of inflammatory cells in local lesion. Excessively elevated levels of chemokines in serum can recruit neutrophils, eosinophils, macrophages, and monocytes in the airway, then resulting in airway responsiveness increase and airway inflammatory cell infiltration\(^{[1,2]}\). Interleukins IL-6 and IL-8 are endogenous chemokines with clear chemotactic function on neutrophils, and can recruit neutrophils in the airway\(^{[3]}\); Eotaxin, also called eosinophil chemotactic factor, can cause eosinophil infiltration in the airway and large synthesis of eosinophil cationic protein, then resulting in airway mucosa injury\(^{[4]}\); MDC is also called macrophage-derived chemokine. MCP1 is a member of monocyte chemoattractant protein family, and can induce infiltration of monocytes and macrophages in the airway\(^{[5]}\); RANTES, also called CCL5, is a chemokine that can regulate activation and secretion function of T cells. It can not only regulate T cell differentiation and promote cytokine secretion, but also activate eosinophils and increase toxic protein release\(^{[6]}\). In the above research, the research group detected serum chemokine contents of bronchial asthma children. Statistical analysis showed that compared with serum index of control group, serum IL-6, IL-8, Eotaxin, MDC, MCP1 and RANTES contents of observation group were higher, which indicated that increased contents of serum chemokines were related to the occurrence of bronchial asthma.

After a large number of inflammatory cells are recruited in the airway, intercellular adhesion is needed for their local infiltration. Adhesion molecule families are important molecules that mediate intercellular adhesion. They play an important role in mutual adhesion processes of inflammatory cells with vascular endothelial cells and bronchial epithelial cells. Integrin is also called integrin family. Its family molecules consist of two subunits, \(\alpha\) and \(\beta\), exist on surfaces of a variety of inflammatory cells, complete intercellular adhesion function through identification of ligands on cell surfaces, and are important adhesion molecules in the body\(^{[7]}\). Integrin \(\alpha_{\alpha}\beta_1\) exists on surfaces of activated eosinophils, and can mediate adhesion of eosinophils to airway epithelium and vascular epithelium through combination with ligand P-selectin as well as VCAM-1\(^{[8,9]}\); Mac-1 is integrin that specifically exists on the surface of neutrophils, and can mediate infiltration of neutrophils in the airway through combination with airway epithelial surface ligand ICAM-1 and CD23\(^{[10,11]}\). \(\alpha_\alpha\beta_6\) is the main type of integrin on the surface of mast cells. Its ligand type is not yet clear. But animal experiments have confirmed that \(\alpha_{\alpha}\beta_6\) is the key molecule that regulates the activation and infiltration of mast cells. Induced sputum specimen can directly reflect conditions of local airway lesion. The research group collected induced sputum specimen and detected integrin molecule and related ligand contents to reflect adhesion molecule contents. Statistical analysis showed that compared with induced sputum index of control group, induced sputum integrin \(\alpha_{\alpha}\beta_1\), \(\alpha_{\alpha}\beta_6\) and Mac-1 as well as its ligand VCAM-1, ICAM-1 and CD23 contents of observation group were higher, which indicated that increased contents of induced sputum integrin molecules and their ligands were related to the occurrence of bronchial asthma.

Immune dysfunction is one of the important mechanisms of bronchial asthma occurrence. Regulatory T cell, \(i.e\). Treg is an important T cell subgroup that regulates immune function and participates in asthma occurrence. It can inhibit the function of immune-effector cells; CD4\(^+\)CD25\(^-\)CD127\(^{low}\) is an important sign on regulatory T cell surface. When CD4\(^+\)CD25\(^-\)CD127\(^{low}\) cell content decreases, inhibitory effect on immune-effector cells weakens, then resulting in proliferation of immune-effector cells and large synthesis of cytokines\(^{[12]}\). Th9 and Th17 are two types of T cell subgroups that are regulated by Treg. They can synthesize and secrete two types of inflammatory factors, IL-9 and IL-17 respectively, and participate in inflammatory response of eosinophils\(^{[13]}\). Follicular T helper cells, \(i.e\). Tfh cell is a newly discovered type of CD4\(^+\)T cell subgroup. CD4 and CXCR5 are important markers on Tfh surface. CD4\(^+\)CXCR5\(^+\)Tfh

<table>
<thead>
<tr>
<th>Group</th>
<th>Treg cell (%)</th>
<th>Th9 cell (%)</th>
<th>Th17 cell (%)</th>
<th>Tfh cell (%)</th>
<th>NK cell (%)</th>
</tr>
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<tbody>
<tr>
<td>Observation</td>
<td>3.19±0.52</td>
<td>9.34±0.98</td>
<td>7.68±0.89</td>
<td>19.14±2.32</td>
<td>8.29±0.91</td>
</tr>
<tr>
<td>Control</td>
<td>5.92±0.81</td>
<td>3.58±0.57</td>
<td>2.91±0.35</td>
<td>9.25±1.04</td>
<td>15.52±1.84</td>
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<td>(P)</td>
<td>&lt;0.05</td>
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cell content can reflect Tfh content; this type of cells can produce antibodies through the auxiliary B lymphocytes to participate in asthma occurrence[14]. NK cells are a kind of innate immune cells. A lot of them head to the inflammatory region in the airway when asthma occurs, with the expression of decreased content in peripheral blood[15]. The research group collected peripheral blood specimen, incubated marker molecules on surfaces of different immune cells and then detected their contents. Statistical analysis showed that compared with peripheral blood immune molecule contents of control group, peripheral blood CD4+CD25+CD127-low Treg and NK cell contents of observation group were lower; Th9, Th17 and Tfh cell contents were higher, which indicated that abnormal contents of peripheral blood immune molecules were related to the occurrence of bronchial asthma.

In conclusion, increased contents of serum chemokines, induced sputum integrin molecules and their ligands, and abnormal contents of peripheral blood immune molecules are related to the occurrence of bronchial asthma.

Conflict of interest statement

We declare that we have no conflicts of interest.

References


