Expression of interleukin 17 and IgE, and its significance in patients with bronchial asthma

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To investigate the expression and its significance of interleukin-17 (IL-17) and immunoglobulin E (IgE) in patients with bronchial asthma, sixty adult patients with bronchial asthma and 30 healthy human control were sequentially enrolled in this study. The production of IL-17 was measured by enzyme-linked immunosorbent assay (ELISA) and fluorescent quantitative polymerase chain reaction (qPCR), respectively. The correlation between expression of IgE and IL-17 was analyzed. It was found that IL-17 was significantly up-regulated in bronchial asthma in comparison to the health control on mRNA level, and serum levels of IL-17 and IgE in bronchial asthma were significantly higher than those in health control, respectively (P < 0.001). Significant positive correlation in bronchial asthma were found between expression of IL-17 and IgE (r = 0.7082, P = 0.0418). The present study implied that expressions of IL-17 and IgE play an important role in the development of bronchial asthma.

Key words: Bronchial asthma, immunoglobulin E, interleukin-17, enzyme-linked immunosorbent assay (ELISA).

INTRODUCTION

Bronchial asthma is one of the most common chronic inflammatory diseases affecting children and young adults and has high morbidity and mortality (Anderson, 2008; Lemanske et al., 2010), and increased total serum immunoglobulin E (IgE) levels (Boushey et al., 1980; Burrows et al., 1989; Kashiwakura et al., 2011), both of which have a strong genetic component (Marsh et al., 1981; Hopp et al., 1990; Postma et al., 1995; Xu et al., 2000; Palm et al., 2012). These features stem largely from the actions of CD4+ Th2 cells, which produce the cytokines IL-4, IL-5, and IL-13 and thereby promote IgE production, eosinophilia, and mucus secretion into the airway (Herrick et al., 2003; Larche et al., 2003). Recent evidence suggests that interleukin-17A (IL-17A) production by TH17 cells or macrophages can also contribute to allergic asthma (Kaminska et al., 2009; McKinley et al., 2008; Molet et al., 2001; Song et al., 2008; Wilson et al., 2009). In addition, it has been demonstrated that T helper type 2 (Th2) and TH17 responses can act synergistically to promote bronchial asthma (Wilson et al., 2009; Kudo et al., 2012). These reports showed that Th17 and Th2 play important roles in bronchial asthma disease. Therefore, an improved understanding of the cellular and molecular mechanisms that regulate TH2 and TH17 effector responses might lead to novel therapeutic strategies to prevent or control bronchial asthma.

Thus, in this study, the expression of IL-17 mRNA level and protein level in serum of the patients with psoriasis was detected by fluorescent quantitative polymerase chain reaction (qPCR) and enzyme-linked immunosorbent assay (ELISA), respectively, and the production of IgE was measured by ELISA. The correlation between expression of IgE and IL-17 was also analyzed.

MATERIALS AND METHODS

Study population

Sixty patients with bronchitis asthma and thirty healthy volunteers were recruited from the Department of Respiratory at The First Affiliated Hospital of Jilin University from January 2010 to March 2012. Informed written consent was obtained from all subjects, and...
Table 1. Expression level of IL-17 mRNA in patient with bronchial asthma and healthy control.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number (n)</th>
<th>Mean ± SD (copy/μg RNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma</td>
<td>60</td>
<td>148032.64 ± 56724.75&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>30518.98 ± 9432.86&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different letter represents the significant difference at p<0.05.

Table 2. Expression of IL-17 and IgE in serum of bronchial asthma and healthy control.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number (n)</th>
<th>IgE (IU/ml)</th>
<th>IL-17 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma</td>
<td>60</td>
<td>247.44 ± 56.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>114.12 ± 21.78&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>45.89 ± 12.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.76 ± 4.92&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different letter represents the significant difference at p<0.05.

this study was approved by the ethics committee of Jilin hospital.

Collection of serum

Briefly, 5 ml venous blood samples were collected on sterile plane tube and were allowed to stand for 30 min at room temperature then centrifuged at 300 g for 5 min. Sera was immediately separated and stored at -20°C until the time of analysis.

RNA preparation and quantitative RT-PCR

Total RNA was isolated from frozen thyroid tissue using blood RNA extraction kit (Tiangen, China) according to the manufacturer’s protocol and as described in the online supplement. The quality and quantity of the RNA was verified by the presence of two discrete electropherogram peaks corresponding to the 28S and 18S rRNA at a ratio approaching 2:1. Using mRNA as template, single-stranded cDNAs were generated by Superscript II reverse transcriptase (Invitrogen) according to the manufacturer’s directions. Real-time quantitative polymerase chain reaction (PCR) experiments were conducted with an ABI Prism 7900 sequence-detection system (Applied Biosystems, Foster City, CA) and SYBR Green PCR Master Mix according to the manufacturer’s protocol. The primer sequences of IL-17(Genebank Accession NO.NM-022190) were as follows: forward, 5′- GAAGGCAGGAATCACAAAT-3′; reverse, 5′- CCGACGGACACCAGT-3′. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Applied Biosystems) served as the internal control. The thermal cycling conditions were as follows: 2 min at 95°C, followed by 40 cycles of 95°C for 15 s and 60°C for 45 s. The expression of interest genes were determined by normalization of the threshold cycle (Ct) of these genes to that of the control GAPDH.

ELISA analysis

To detect protein expression of IL-17 and IgE, ELISA was conducted using assay kit. The human IL-17 enzyme-linked immunosorbent assay (ELISA) kit (Huamei, China) is an in vitro ELISA for the quantitative measurement of human IL-17 in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human IL-17 coated on a 96-well plate. Standards and samples are pipetted into the wells and IL-17 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human IL-17 antibody is added. After washing away the unbound biotinylated antibody, Horseradish Peroxidase (HRP)-conjugated streptavidin is pipetted to the wells. The wells are again washed, a 3,3′,5,5′-tetramethylbenzidine (TMB) substrate solution is added to the wells and color is developed in proportion to the amount of IL-17 bound. The stop solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm (Ray Biotech, Merk, Germany). The human IgE enzyme-linked immunosorbent assay (ELISA) kit (Huamei, China) is a quantitative measurement of human IgE cell lysate and tissue lysate. The IgE was detected according to the manufacturer’s protocol.

Statistics analysis

To calculate the statistical differences between the control and bronchitis asthma, the Statistical Package SPSS 16.0 (SPSS Incorporated, Chicago) was used for all analysis. Student’s t test was used to determine the significance of differences among the groups. Correlation between IL-17 and IgE expression was analyzed using Spearman’s rank correlation analysis. All values were expressed as mean ± standard deviation (SD). In general, p values less than 0.05 were considered statistically significant.

RESULTS

Fluorescent quantitative RT-PCR analysis of IL-17 expression

In order to detect the mRNA expression of IL-17 in patients with bronchitis asthma, fluorescent quantitative RT-PCR (qPCR) was conducted. As shown in Table 1, IL-17 mRNA levels in bronchitis asthma group were significantly increased compared to health control group (P<0.05), which showed that IL-17 mRNA expression increased in patients with bronchitis asthma.

ELISA analysis of IL-17 expression

In order to further confirm IL-17 protein expression in serum, ELISA was conducted. The results showed that there were significant differences in IL-17 expression between bronchitis asthma and healthy control (P<0.05). The IL-17 protein expression levels in bronchitis asthma group were significantly up-regulated as compared to health control group (P<0.05) (Table 2), which corroborates with the result of IL-17 mRNA expression increasing in patients with bronchitis asthma.

Correlation between IL-17 and IgE in bronchitis asthma

In order to detect IgE protein expression in bronchitis IgE expression levels in bronchitis asthma group were significantly higher than those of health control group (P<0.05). Moreover, the correlation between expression of IgE and IL-7 was also analyzed in this study by Spearman’s rank correlation analysis. It was found that...
the mean production of IL-17 in bronchitis asthma was significantly positively correlated to the production of IgE \((r=0.7082, P<0.05)\).

**DISCUSSION**

Bronchial asthma is a kind of airway chronic inflammatory disease, which is characterized by the involvement of inflammatory cells, mediators of inflammation and neurotransmitters (Lloyd et al., 2009) and its pathogenesis is not fully understood so far (Louis et al., 2012). At present, IgE is thought to play an important role in the bronchial asthma (Palm et al., 2012). Therefore, in clinical practice, testing for allergen-specific IgE is used in the diagnosis of asthma and to guide therapy, including environmental modification and immunotherapy. Interference with IgE function has also recently become a focus of pharmacologic therapy (Warrington, 2010).

In the present study, we found that that IgE expression levels in bronchitis asthma group were significantly increased in comparison to healthy control group \((P<0.05)\) by ELISA detection, which was in agreement with previous study that showed that IgE expression elevated in asthma (Boushey et al., 1980; Burrows et al., 1989). The role of IL-17 and TH17 cells in human asthma is still unclear; however, there are some evidence suggesting their involvement in the disease. Zhao et al. (2010) investigated TH1, TH2, and TH17 cell subsets by flow cytometry as well as cytokines from peripheral blood mononuclear cells (PBMC) from allergic asthmatics and controls. The results showed that there was a significant increase of IL-17 in the asthmas compared with healthy controls, which is in agreement with our results that IL-17 expression levels in bronchitis asthma were significantly up-regulated in comparison to healthy control group \((P<0.05)\). Therefore, we suggested that IL-17 might play an important role in development of asthma.

In this study, it was found that the mean production of IL-17 in bronchial asthma was significantly correlated to production of IgE, and was positive \((r=0.7082, P<0.05)\). The reason might be that IL-17 plays important direct role in creating proinflammatory and chemotactic environment, enhances IL-6, IL-8 and ICAM-1 expression by keratinocyte, promotes lymphocyte infiltration within epidermis, at the same time promotes more rapid recruitment of neutrophil through induced chemokine expression (Lowes et al., 2008; Zheng et al., 2007) and drives the allergic TH2 response, which produces the cytokines IL-4, IL-5, and IL-13 and thereby promotes IgE production, eosinophilia, and mucus secretion into the airway (Herrick et al., 2003; Larche et al., 2003). Our results implied that IL17 could promote IgE production in serum of patients with bronchitis asthma, which helps in the control and treatment of bronchitis asthma.

In summary, our study reveals that the expression of IL-17 and IgE was higher in patients with bronchial asthma than that of healthy control and there were correlation between IL-17 and IgE, indicating that IL17 and IgE in bronchial asthma was the proximal regulator in its pathogenesis, which may be efficacious in targeting the reduction of IL-17 and IgE expression for the treatment of bronchial asthma.

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