Interleukin-12 and Peripheral Blood Invariant Natural Killer T Cells as an Axis in Childhood Asthma Pathogenesis

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ABSTRACT

Interleukin-12 (IL-12) is a key cytokine involved in regulating the balance between TH1 and TH2 cells by promoting TH1 response. A reduced capacity to produce this cytokine could lead to aberrant TH2 development. On the same aspect significant impact of IL-12 on invariant natural killer T (iNKT) cells was reported. Therefore, we examined the serum levels of IL-12 and the absolute number of peripheral blood iNKT cells from 37 children with controlled asthma and 11 normal controls (age-matched) and correlating these two parameters with clinical asthma severity and Pulmonary function tests (PFTs).

A significant decrease of serum levels of IL-12 and peripheral iNKT cells was found in total asthmatic cases compared with normal controls. This significant decrease of IL-12 levels was observed in severe asthmatic patients compared with mild and moderate cases.

Serum levels of IL-12 and the numbers of peripheral iNKT cells were positively correlated with PFTs in both total asthmatic groups and in children with severe persistent asthma.

Inverse correlation was found between serum level of IL-12 and different degrees of asthma. Whereas the numbers of peripheral blood iNKT cells showed no significant difference between clinical asthma severities.

Impaired IL-12 production in asthmatic children beside decreasing the number of peripheral blood iNKT cells could be considered as a key component in asthma pathogenesis and hence their therapeutic manipulation may be of help in asthma management.

Key words: Asthmatic children; Clinical asthma severity; invariant Natural killer T (iNKT) cells; IL-12; Peripheral blood; Pulmonary function tests

INTRODUCTION

Asthma is a disease characterized by eosinophilic airway inflammation, bronchospasm, and airway hyperreactivity. It has become clear that both the pathogenesis and the severity of asthma are mediated through T helper type 2 (TH2) lymphocytes and compensatory decrease of T helper type 1 (TH1) lymphocytes.¹

Interleukin-12 (IL-12) can induce interferon-γ (IFN-γ) secretion in activated, invariant natural killer T (iNKT) cells, natural killer cells, augment natural killer-cell mediated cytotoxicity, and facilitate the expansion of TH1-biased CD4 and CD8 T effector cells.²,³

Dendritic cells are a major source of IL-12 and are thought to be major regulators of T-cell TH1 or TH2
polarization. Interaction of dendritic cells and iNKT cells leads to the activation of iNKT cells, and secretion of many cytokines such as IL-4 and granulocyte–macrophage colony-stimulating factor that are important for the differentiation of myeloid dendritic cells. Collectively, these findings suggest that iNKT-cell and dendritic cell cross talk is critical for shaping the subsequent adaptive immune response. Thus the source and quantity of IL-12 is expected to have a significant impact on the activation of iNKT cells.

Invariant natural killer T (iNKT) cells are component of innate immunity, they secret huge amounts of TH1 cytokines immediately after activation. The influence of peripheral blood iNKT cells in the severity of experimental allergic asthma was documented. In mouse models of atopic asthma, the pivotal role of iNKT cells in the development of eosinophilic airway inflammation and airway hyperresponsiveness (AHR) were found to be essential. However, the role of peripheral blood iNKT cells in the pathogenesis of human asthma remains controversial. The relationship between airway inflammation and iNKT cells was documented in experimental animals.

The aim of this study was to investigate the changes of serum levels of IL-12 and peripheral blood iNKT cells in controlled asthmatic children in relation to clinical asthma severity and pulmonary function tests (PFTs).

MATERIALS AND METHODS

Subjects
A total of 37 persistent asthmatic children (mild, moderate and severe cases) and 11 normal controls (age-matched) children were included in the study. Normal controls had no respiratory symptoms or history of allergic diseases. Asthmatic children who were included in this study, characterized by typical asthma symptoms, improvement in the prebronchodilator FEV1 of ≥12% after administration of salbutamol (200 μg), and positive skin prick test.

Asthma severity was classified according to the Global Initiative for Asthma guideline (GINA) 2002 as intermittent or persistent (mild, moderate and severe). The atopic status was demonstrated by means of positive immediate skin prick responses to at least one of 10 common inhalant allergens (skin wheal response of >5mm at 15 minutes, considered positive). The local ethical committee approved the study, and the parents gave written informed consent.

Skin Prick Tests
Skin prick tests were performed only to asthmatic group with different antigens, including house dust mites (Dermatophagoides pteronyssimus and Dermatophagoides farinae), cat and dog epithelial cells, and mold and pollen antigens (Omega, Canada), together with negative (saline) and positive (0.5% histamine HCl) controls. A mean weal diameter of ≥3 mm at 15 min was defined as a positive response. Children were considered atopic if they had at least one positive skin prick test response.

Pulmonary Function Testing
Pulmonary Function Tests (PFTs) were performed by Master Screen Body (Typ.B/IEC.601-1/IPZO) for measurement of static and dynamic pulmonary functions. Three maneuvers were done including slow spirometry, forced spirometry, and maximal voluntary volume maneuvers.

Estimation of IL-12
IL-12P40 Enzyme-linked immunosorbant assay (ELISA) (Diaclon Research Company (Besancon Cedex, France)) was used for estimation of IL-12.

Evaluation of Peripheral Blood iNKT Cells
Enumeration of iNKT cells was done by flow cytometry: fluorescent-labelled monoclonal antibodies (CD16/CD56 phycoerthrin) (Clonab, Frankfurt, Germany), were used to identify iNKT cells.

Statistical Analysis
Statistical analysis was done using SPSS (Statistical Package for Social Science) software (version 12.0, SPSS Inc, Chicago, IL). Data were presented as mean±Standard Deviation (SD). Differences between values were determined using: Kendall Rank Correlation Coefficient, and Spearman Rank correlation coefficient. Data of the groups were analysed using Student's t-test. A value of p<0.05 was regarded as a significant difference.

RESULTS
Patients and normal controls characteristics are shown at Table 1.
Table 1. Characteristics of the studied subjects.

<table>
<thead>
<tr>
<th>Topics</th>
<th>Total asthmatic children</th>
<th>Mild persistent asthma</th>
<th>Moderate persistent asthma</th>
<th>Severe persistent asthma</th>
<th>Normal controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>37</td>
<td>15</td>
<td>7</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>Age in years</td>
<td>10.17±1.69</td>
<td>11.13±2.41</td>
<td>9.28±1.49</td>
<td>10.3±1.6</td>
<td>11.08± 2.31</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>24/13</td>
<td>10/5</td>
<td>5/2</td>
<td>9/6</td>
<td>6/5</td>
</tr>
<tr>
<td>Interleukin-12 p40 (pg/ml)</td>
<td>98.66±50.0*</td>
<td>114.55±49.81</td>
<td>113.73±22.74</td>
<td>69.68±33.98*</td>
<td>164.7±26.21</td>
</tr>
<tr>
<td>Total iNKT cell (number/ml)</td>
<td>19.58±6.28*</td>
<td>18.71±7.18</td>
<td>22.14±8.72</td>
<td>21.33±5.77</td>
<td>43.08±5.14</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD
Student t test between each group was used,
*P < 0.05 significant

Serum levels of IL-12 and the absolute number of peripheral blood iNKT cells were significantly decreased in total asthmatic cases when compared with normal controls. There was a significant decrease of the serum IL-12 levels among severe persistent asthmatic group compared with mild and moderate patients. On the other hand, circulating peripheral blood iNKT cells showed insignificant difference among the three asthmatic subgroups (Table 1).

Negative correlation was found between different degrees of asthma severity and serum levels of IL-12 (r=-0.77, p<0.001). While this finding was not detected as regard the peripheral blood iNKT cells (r=-0.02, p=0.87).

Our study showed a significant positive correlations between PFTs with both serum IL-12 and peripheral blood iNKT cells for total asthmatics cases, and patients with severe persistent asthma (Table 2).

DISCUSSION

Asthma is a chronic airway inflammation characterized by episodes of reversible airway obstruction, AHR, IgE production, increased mucus secretion, and an airway infiltrate with eosinophilic granulocytes, mast cells, and lymphocytes. This airway inflammation is caused by a defect in immune regulation involving T helper lymphocytes, with an increase in TH2 lymphocytes and a compensatory decrease in Th1 lymphocytes.

Table 2. Correlation between serum levels of Interleukin-12 (IL-12) (pg/ml) and peripheral blood iNKT (Number/ml) cells with pulmonary function tests (PFTs) in the studied groups

<table>
<thead>
<tr>
<th>PFTs</th>
<th>Total asthmatic children</th>
<th>Mild persistent asthma</th>
<th>Moderate persistent asthma</th>
<th>Severe persistent asthma</th>
<th>Normal controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IL-12</td>
<td>iNKT</td>
<td>IL-12</td>
<td>iNKT</td>
<td>IL-12</td>
</tr>
<tr>
<td></td>
<td>r(p)</td>
<td>r(p)</td>
<td>r(p)</td>
<td>r(p)</td>
<td>r(p)</td>
</tr>
<tr>
<td>FEV1%</td>
<td>0.58(0.001*)</td>
<td>-0.01(0.52)</td>
<td>0.29(0.29)</td>
<td>0.37(0.19)</td>
<td>0.49(0.21)</td>
</tr>
<tr>
<td>FEF%</td>
<td>0.41(0.013*)</td>
<td>1.03(0.54)</td>
<td>-0.23(0.41)</td>
<td>0.24(0.39)</td>
<td>0.37(0.41)</td>
</tr>
<tr>
<td>FEF 25%</td>
<td>0.33(0.046*)</td>
<td>0.13(0.41)</td>
<td>-0.23(0.42)</td>
<td>0.21(0.45)</td>
<td>0.28(0.54)</td>
</tr>
<tr>
<td>FEF 50%</td>
<td>0.48(0.003*)</td>
<td>0.16(0.33)</td>
<td>-0.07(0.79)</td>
<td>0.28(0.31)</td>
<td>0.49(0.259)</td>
</tr>
<tr>
<td>FEF 75%</td>
<td>0.54(0.034*)</td>
<td>0.62(0.012*)</td>
<td>-0.05(0.86)</td>
<td>0.19(0.51)</td>
<td>0.38(0.39)</td>
</tr>
</tbody>
</table>

Spearman rank correlation coefficient was used.
* P<0.05 significant.
Compared with normal controls, significant decrease of serum levels of IL-12 and the number of peripheral blood iNKT cells were found in total asthmatic cases. Children with severe persistent asthma showed a significant decrease of serum levels of IL-12 compared with mild and moderate cases. Whereas, the number of peripheral blood iNKT cells did not differ in regard to the clinical severity of asthma. In adult patients with allergic asthma, a decrease of circulating peripheral blood iNKT cells had been reported. The mechanism for the low level of peripheral blood iNKT cells is still unclear at present. This decrease may be due to migration of iNKT cells from the periphery to inflammatory sites in the lung, which is supported by Pham-Thi et al. who demonstrated that iNKT cells are increased in bronchoalveolar lavage fluid of children with asthma. Also, Oishi, et al. in human (healthy donors) showed that the activated iNKT cells predominately produce a large amount of IFN-γ but not IL-4. Another possibility is that, endogenous IL-12 was found to have an effect on NK-T cells which may play an essential role in the effector phase of allergic asthma. Also, IL-12 has been shown to stimulate NKT cells, and blockade of IL-12 may have led to reduced NKT cell activity. These observations may indicate that the decrease of IL-12 and peripheral iNKT cells in asthmatic children could facilitate the development of allergic asthma.

In our study, a significant positive correlations of PFTs was found with both serum IL-12 and peripheral blood iNKT cells for total asthmatics cases, and children with severe persistent asthma. Also a negative correlation was found between different degrees of asthma severity and serum levels of IL-12 which was not detected with peripheral blood iNKT cells.

In consistence with our results, endogenous IL-12p40 have been demonstrated to have an essential role for inhibition of AHR in a murine asthma model with prolonged antigen exposures, and also, depletion of IL-12-producing dendritic cells was found to abrogate the characteristic features of airway inflammation.

On the Other hand, Our finding is against what has been reported in experimental models in which the activation of peripheral blood iNKT cells has been shown to be sufficient to induce AHR. Also in mice deficient for peripheral blood iNKT cells, allergen-primed and challenged mice, could not develop cardinal features of asthma such as AHR and eosinophilic airway inflammation. Whereas in human, iNKT cells upon activation they behave like TH1, therefore their decrease in the peripheral blood may facilitate TH2 immune response and hence development of allergic asthma.

**CONCLUSION**

Impaired IL-12 production in asthmatic children beside the decreasing the number of peripheral blood
Interleukin-12 and Peripheral Blood Invariant Natural Killer T Cells

Invariant natural killer T (iNKT) cells it could be considered as a key component in asthma pathogenesis and hence their therapeutic manipulation may be of help in asthma management.

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REFERENCES