**SHORT REPORT**

Enhanced peripheral leukocyte leukotriene production and bronchial hyperresponsiveness in asthmatics


ABSTRACT: Leukotrienes (LTs) are pro-inflammatory mediators that contribute to the pathophysiological features of asthma. The relationship between the amounts of LTB4 and LTC4 produced by the leukocytes of asthmatic patients on the one hand and immunoglobulin E (IgE)-mediated allergy, asthma exacerbations and bronchial hyperresponsiveness was studied.

Leukocytes were obtained from peripheral blood drawn from 29 atopic and 27 nonatopic asthmatics during exacerbations and clinically controlled periods, as well as from 20 control individuals. The leukocytes were stimulated with calcium ionophore A23187 to induce LTB4 and LTC4 production. Allergy was assessed by means of specific serum IgE or by positive skin tests, whereas bronchial hyperresponsiveness was measured by methacholine challenge.

The leukocytes of the asthmatics generated significantly more LTB4 (p<0.05) and LTC4 (p<0.01) than those of controls. The leukocytes of patients with atopic asthma generated significantly more LTC4 than those of patients with nonatopic asthma (p<0.01). Significantly more LTC4 was produced by leukocytes obtained during exacerbations, than by those obtained during clinically controlled periods (p<0.01). In addition, there was a significant correlation between LTB4 generation by leukocytes and the degree of bronchial hyperresponsiveness to methacholine (r=-0.792, p<0.0001).

These results suggest that leukotriene C4 production by leukocytes is associated with immunoglobulin E-mediated allergy and asthma exacerbations, and further that generation of leukotriene B4 is closely related to bronchial hyperresponsiveness in patients with asthma.

Asthma is characterized by airway inflammation. Inflammatory cells such as lymphocytes, neutrophils and eosinophils, and a number of cytokines including leukotrienes (LTs) released from these cells, participate in the pathophysiological changes in the airways of patients with asthma [5]. Cysteinyl LTs (cys-LTs) induce bronchoconstrictor effects [6], increase mucus formation [7], and induce bronchial wall oedema [8]. LTB4 stimulates neutrophil chemotaxis [9] and activates those cells, leading to the release of mediators and superoxides.

The present study assesses the relationship between the amounts of LTC4 and LTB4 generated by the peripheral leukocytes of patients with asthma and immunoglobulin E (IgE)-mediated allergy, asthma attacks and bronchial hyperresponsiveness.

Patients and methods

Patients

Fifty-six patients with asthma (36 female and 20 male; mean age 58 yrs) and 20 control individuals (11 female and 9 male; mean age 58 yrs), all of whom were lifetime non-smokers were examined. Asthma was diagnosed according to the criteria of the American Thoracic Society [10]. All patients with asthma were symptomatic, with episodic dyspnoea, wheezing and cough, and showed evidence of ≥15% reversibility of forced expiratory volume in one second FEV1 after inhalation of 200 μg salbutamol. The normal controls had no history of wheeze or allergic diseases and their spirometric data were normal. Total and specific serum IgE were measured using the Pharmacia CAP System® (Pharmacia Diagnostics AB, Uppsala, Sweden). Diagnosis of atopy was based on specific serum IgE and/or a positive skin test for common inhalant allergens.

All patients had been inhaling β2-agonists as needed (table 1). Ten of the 29 atopic asthmatic patients had chronic severe asthma and had been on oral glucocorticoid therapy for >2 yrs (steroid-dependent intractable
Table 1. – Characteristics of controls and asthmatic patients

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Atopic asthma</th>
<th>Nonatopic asthma</th>
</tr>
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<tbody>
<tr>
<td>Participants n</td>
<td>20</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td>Male/female n</td>
<td>9/11</td>
<td>7/12</td>
<td>4/6</td>
</tr>
<tr>
<td>Age yrs*</td>
<td>58±16</td>
<td>57±12</td>
<td>60±10</td>
</tr>
<tr>
<td>Serum IgE U·mL⁻¹⁻¹*</td>
<td>34±2</td>
<td>23±3**</td>
<td>26±3**</td>
</tr>
<tr>
<td>Specific IgE n</td>
<td>0</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td>FVC % pred</td>
<td>108.5±7.7</td>
<td>100.5±18.0</td>
<td>95.0±15.9</td>
</tr>
<tr>
<td>FEV1 % pred</td>
<td>99.1±4.4</td>
<td>88.6±18.8</td>
<td>70.5±19.4*</td>
</tr>
<tr>
<td>FEV1/FVC %</td>
<td>82.0±6.3</td>
<td>68.1±9.8*</td>
<td>55.5±9.9***</td>
</tr>
<tr>
<td>Medication n</td>
<td>0</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Systemic corticosteroids</td>
<td>0 , 0</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Oral β₂-agonists</td>
<td>0</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Theophylline</td>
<td>0</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td>Inhaled corticosteroids</td>
<td>0</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Sodium cromoglycate</td>
<td>0</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>LT generation ng·5 x 10⁶ leukocytes⁻¹</td>
<td>43±24.4</td>
<td>83.2±31.4*</td>
<td>57.7±30.7</td>
</tr>
<tr>
<td>LTC₄</td>
<td>5.3±6.0</td>
<td>48.7±21.2**</td>
<td>8.4±5.8*</td>
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<tr>
<td>SDIA -</td>
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<td>SDIA +</td>
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<td>SDIA -</td>
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<td>SDIA +</td>
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</table>

Data are presented as absolute values or mean±sd. *: geometric mean±sd. SDIA+: steroid-dependent intractable asthma (SDIA)-positive; SDIA-: SDIA-negative; IgE: immunoglobulin E; FVC: forced vital capacity; FEV1: forced expiratory volume in one second; LT: leukotriene. **: p<0.05, p<0.01 versus controls; ***: p<0.05, p<0.01 versus SDIA-atopic asthmatics; 11: p<0.05, p<0.01 versus SDIA-asthmatics.

Results are expressed as mean±sd. Serum IgE levels are given as the geometric mean and geometriso of the mean. This is the analog of the so of the log10-transformed serum IgE levels. Groups were compared by one-way analysis of variance (ANOVA).
variance for multiple comparisons. The amounts of LTB₄ and LTC₄ released by leukocytes during escalation and clinically controlled periods were compared using a paired t-test. Relationships between LT generation and log₁₀ \( D_{\text{min}} \) were tested by linear regression analysis.

A p-value of <0.05 was regarded as significant.

**Results**

The baseline characteristics of the controls and asthmatic patients are presented in table 1. The type and dosage of medication did not differ among the asthmatic groups. Serum IgE levels did not differ significantly between patients with SDIA and non-SDIA in either the atopic or nonatopic group. The FEV₁ and FEV₁/forced vital capacity (FVC) did not differ significantly between atopic and nonatopic patients by leukocytes from asthmatics.

Table 1 shows the amount of LTB₄ and LTC₄ generated by the peripheral leukocytes of asthmatic patients and control individuals on stimulation with the calcium ionophore A23187. The leukocytes of atopic and nonatopic asthmatics who were not receiving glucocorticoid therapy generated significantly more LTB₄ \((p<0.05)\) and LTC₄ \((p<0.01)\) than those of controls. In contrast the production of either LTB₄ or LTC₄ by leukocytes of patients with atopic or nonatopic asthma treated with long-term systemic glucocorticoid therapy (SDIA) and those of controls did not differ significantly. Among asthmatics who were not receiving oral glucocorticoid therapy, significantly more LTC₄ was produced by the atopic than by the nonatopic asthmatic patients \((p<0.01)\). The leukocytes of atopic asthmatics who were not on an oral glucocorticoid regimen generated significantly more LTC₄ than those of atopic patients with SDIA \((p<0.01)\). The amounts of LTB₄ generated by patients with and without SDIA did not differ significantly.

The amount of LTB₄ generated by leukocytes of atopic asthmatic patients was 84.6±51.6 ng · 10⁶ cells⁻¹ during exacerbations, and 72.5±30.0 ng · 10⁶ cells⁻¹ when clinically controlled. Nonatopic patients generated 85.9±34.5 ng · 10⁶ cells⁻¹ during exacerbations, and 75.9±31.6 ng · 10⁶ cells⁻¹ when clinically controlled. The amount of LTB₄ generated during either period did not differ significantly between atopic and nonatopic asthmatics (fig. 1a).

Figure 1b shows the production of LTC₄ by the leukocytes of patients with atopic and nonatopic asthma in relation to asthma exacerbations. Significantly more LTC₄ was produced during the exacerbation period than when clinically controlled, by both the atopic and nonatopic asthmatics. The amount of LTC₄ generated by leukocytes from atopic asthmatics increased from 49.9 ng · 10⁶ cells⁻¹ when clinically controlled, to 72.5 ng · 10⁶ cells⁻¹ during the exacerbation period \((p<0.01)\). The amount of LTC₄ produced by nonatopic asthmatics also increased significantly from 22.9 ng · 10⁶ cells⁻¹ when clinically controlled, to 60.8 ng · 10⁶ cells⁻¹ during the exacerbation period.

The relationship between the levels of LTB₄ and LTC₄ produced by leukocytes and bronchial hyperresponsiveness to methacholine in asthmatics who were not receiving oral glucocorticoid therapy when they were clinically controlled were examined. Figure 2a shows a highly significant correlation between the level of LTB₄ generation and log₁₀ \( D_{\text{min}} \) \((r=-0.792,\ p<0.001)\). When the level of LTC₄ production was plotted against log₁₀ \( D_{\text{min}} \), the correlation was still significant but with a much larger dispersion of data around the regression line \((r=-0.489,\ p=0.0025)\) (fig. 2b).

**Discussion**

LTB₄ and the cysteinyLTs (LTC₄, LTD₄ and LTE₄) play important roles in the pathophysiology of the airways of patients with bronchial asthma. LTB₄ (as well as interleukin 8) acts as a chemoattractant/activator of neutrophils, and causes bronchial hyperresponsiveness and neutrophil accumulation in the airway [15]. Eosinophils and mast cells may synthesize LTC₄, neutrophils synthesize LTD₄, and macrophages may synthesize both LTC₄ and LTB₄ [16]. Increased production of LTC₄ is often accompanied...
by eosinophil accumulation in the airway [17]. The amount of LTC4 produced by eosinophils depends not only on the number of eosinophils but also on their degree of activation [18].

In the present study, the generation of LTB4 and LTC4 by leukocytes stimulated with calcium ionophore A23187 was studied in patients with asthma, in relation to IgE-mediated asthma exacerbations, and bronchial hyperresponsiveness. The results showed that the leukocytes of asthmatics who are not on oral glucocorticoid therapy when they were clinically controlled. Regression lines are shown. 

Fig. 2. – Relationship between: a) leukotriene (LT) B4 (r = -0.792, p=0.0001); and b) LTC4 generation (r=-0.489, p=0.0025) by leukocytes and bronchial hyperresponsiveness to methacholine of patients with atopic (○) and nonatopic asthma (□) who were not on oral glucocorticoid therapy when they were clinically controlled. Regression lines are shown. Dmin: minimum dose of methacholine, i.e. the cumulative dose reached at the inflection point at which the reciprocal of respiratory resistance decreased linearly.

Patients with a history of severe asthma formed more LTC4 than those of patients with less severe disease. The present results also revealed that the leukocytes of patients with atopic asthma who were not receiving oral glucocorticoid therapy generated significantly more LTC4 than those of patients with nonatopic asthma. These results suggest that LTC4 production is more closely related to IgE-mediated allergy than to other asthmatic reactions. However, the leukocytes of patients with atopic or nonatopic asthma generated similar levels of LTB4. Levels of LTB4 generation both during exacerbations and when clinically controlled were similar in both atopic and nonatopic asthmatics.

This study demonstrates that the leukocytes of patients with relatively more severe hyperresponsiveness to methacholine produced significantly more leukotriene B4 than those of patients who are less hyperresponsive. Furumura et al. [15] demonstrated that interleukin-8 induces bronchial hyperresponsiveness as well as airway neutrophil accumulation in guinea-pigs in vivo, and that this may be partly mediated by the release of leukotriene B4. Although hyperresponsiveness to methacholine also significantly correlated with leukotriene C4 production, this correlation was weaker than that between methacholine hyperresponsiveness and LTB4 production. Taken together, the results of the present study suggest that immunoglobulin E-mediated allergy participates predominantly in the production of leukotriene C4, which is enhanced during asthma exacerbations. In contrast, enhanced production of leukotriene B4 might lead to an increase in bronchial hyperresponsiveness in patients with asthma.

References


