Chemical mediator and cellular reaction in the bronchoalveolar lavage fluid of patients with steroid-dependent intractable asthma (SDIA).

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Abstract

The effects of long-term glucocorticoid therapy on chemical mediator and cellular reaction in the airways were examined in 69 patients with bronchial asthma. The histamine release induced by Ca ionophore A23187 from cells in the bronchoalveolar lavage (BAL) fluid of atopic asthmatics was significantly lower in the subgroup with steroid-dependent intractable asthma (SDIA) than in non-SDIA patients (p < 0.05). In contrast, histamine release in nonatopic SDIA patients did not differ from nonatopic non-SDIA patients. The release of leukotriene C4 (LTC4) was significantly lower in atopic patients with SDIA (p < 0.02). However, there was no significant difference in LTC4 release between nonatopic patients with SDIA and without SDIA. The proportion of BAL lymphocytes was significantly lower in atopic patients with SDIA than in those without it (p < 0.05), although there was no significant difference between the nonatopic patients with and without SDIA. These results show that glucocorticoids affect humoral and cellular events in the airways of atopic asthmatics more than in those of nonatopic asthmatics.

KEYWORDS: histamine, leukotrienes, BAL cells, intractable asthma, glucocorticoids

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Chemical Mediator and Cellular Reaction in the Bronchoalveolar Lavage Fluid of Patients with Steroid-Dependent Intractable Asthma (SDIA)

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The effects of long-term glucocorticoid therapy on chemical mediator and cellular reaction in the airways were examined in 69 patients with bronchial asthma. The histamine release induced by Ca ionophore A23187 from cells in the bronchoalveolar lavage (BAL) fluid of atopic asthmatics was significantly lower in the subgroup with steroid-dependent intractable asthma (SDIA) than in non-SDIA patients \( (p < 0.05) \). In contrast, histamine release in nonatopic SDIA patients did not differ from nonatopic non-SDIA patients. The release of leukotriene \( \mathrm{C}_4 \) (LTC\(_4\)) was significantly lower in atopic patients with SDIA \( (p < 0.02) \). However, there was no significant difference in LTC\(_4\) release between nonatopic patients with SDIA and without SDIA. The proportion of BAL lymphocytes was significantly lower in atopic patients with SDIA than in those without it \( (p < 0.05) \), although there was no significant difference between the nonatopic patients with and without SDIA. These results show that glucocorticoids affect humoral and cellular events in the airways of atopic asthmatics more than in those of nonatopic asthmatics.

Key words: histamine, leukotrienes, BAL cells, intractable asthma, glucocorticoids

The onset mechanism of bronchial asthma has early humoral and late cellular phases. In the early humoral phase, chemical mediator like histamine and leukotrienes are released from tissue mast cells \((1-5)\), leading to bronchoconstriction, bronchial wall edema, and mucus hypersecretion. In the late cellular phase, inflammatory cells infiltrate the airways of asthma patients, and these include lymphocytes, neutrophils, eosinophils, and basophils from the peripheral blood \((6-16)\). It is now widely accepted that airway inflammation is a common feature of bronchial asthma, because it is observed even in mild asthma \((17-19)\), and that this inflammation increases the severity and complexity of the disease.

In recent years, many kinds of antiasthma drugs have been developed. However, there are still some patients with intractable asthma that cannot be controlled by the usual antiasthma agents. These individuals require long-term glucocorticoid therapy to control their asthma attacks. It is well known that long-term glucocorticoid therapy causes many adverse effects, and that these drugs have an immunosuppressive action. However, there are few reports about the effects of glucocorticoids on airway inflammation in patients with asthma.

In the present study, the effects of long-term glucocorticoid therapy on the release of chemical mediators from bronchoalveolar lavage (BAL) cells, and on the inflammatory cells themselves were examined in patients with steroid-dependent intractable asthma (SDIA).

Subjects and Methods

The subjects were 69 asthma patients (41 women and 28 men) with a mean age of 53.5 years (range: 21–73 years). The subjects...
were divided into a group who had received glucocorticoid therapy for more than 2 years (steroid-dependent intractable asthma; SDIA) and another group whose asthma attacks were controlled by the usual antiallergic agents (non-SDIA). Asthma was classified as either atopic with IgE antibodies to inhalant allergens or a serum IgE level > 500 IU/ml or nonatopic with a negative skin reaction to various allergens and a serum IgE level < 100 IU/ml.

BAL was performed by a previously reported method (20, 21) when the patients were in the interval between asthma attacks. Informed consent for the BAL examination was obtained from all the subjects. After filtration through a sterile stainless steel mesh, the total number of cells was counted. The filtrates were centrifuged at 1,200 rpm for 10 min at 4°C. Then the cell pellet was resuspended in Tris buffer (containing human serum albumin, Ca and Mg) to assess the release of histamine and leukotrienes C4 and B4 from the cells and to obtain the differential cell counts.

For the experiments on chemical mediator release from BAL cells, the number of cells was adjusted to 10⁶/ml in Tris buffer. Ca ionophore A23187 (1 µg) was added to the cell suspension, and the suspension was incubated for 15 min at 37°C, and the concentrations of histamine (in the supernatant and cells) and leukotrienes C4 and B4 (in the supernatant only) were measured. The histamine concentrations were assayed by an automated spectrophotometric histamine analysis system (Technicon Co.) (22) after perchloric acid precipitation, as previously reported (23, 24). The results were expressed as a percentage of total histamine release. For measurement of LTC4 and B4, the cell suspension was incubated with Ca ionophore A23187, and then 4 ml of 100% ethanol was added. After the suspension was centrifuged, the supernatant was vacuum dried and resuspended in 250 µl of high-performance liquid chromatography (HPLC) solvent (CH3CN/H2O = 1:1). The resuspended solution was subjected to HPLC (c-18 reversed-phase column, with detection at 280 nm), and the results were expressed as ng/10⁶ cells.

The proportions of BAL cells were determined by the differential counting of 500 cells excluding epithelial cells, and the results were expressed as a percentage of the total cell number.

Bronchial reactivity to methacholine was evaluated using an Astograph (TSK 6100H, Chest Co.). Different concentrations of methacholine (49, 98, 195, 390, 781, 1,563, 3,125, 6,250, 12,500 and 25,000 µg/ml) were prepared for bronchial challenge according to the method of Chai et al. (25). The increase of total respiratory resistance (Rrs) after methacholine inhalation was assessed by the oscillation method and the methacholine concentration causing a significant increase in Rrs was defined as the minimum threshold concentration (Cmin). All medications were stopped for 12 h prior to the examination.

Serum corticoid levels were estimated by radioimmunoassay (RIA) at 7 to 8 AM. The serum IgE level was measured by the radioimmunosorbent test (RIST) and IgE antibodies to inhalant allergens were evaluated by the radioallergosorbent test (RAST).

The significant differences in the mean values of the obtained data were estimated using the unpaired Student's t test and p values of < 0.05 were regarded as indicating significance.

Results

Clinical profile of the subjects. The subjects were divided into SDIA and non-SDIA groups, according to their need for glucocorticoids, and were further divided into those with atopic and nonatopic asthma according to the presence or absence of IgE-mediated allergy (Table 1). In the patients with atopic asthma, serum IgE levels were high and IgE antibodies to inhalant allergens were commonly found. In contrast, the serum IgE levels were low and IgE antibodies were not found in any of patients with nonatopic asthma.

Serum corticoid levels were very low in the patients with atopic and nonatopic SDIA. The serum cortisol levels in atopic SDIA patients (3.5 µg/dl) were significantly lower than in atopic non-SDIA patients (10.6 µg/dl) (p < 0.001). The serum cortisol levels were also significantly lower in patients with nonatopic SDIA (2.5 µg/dl) than in those with nonatopic non-SDIA (10.3 µg/dl) (p < 0.001) (Fig. 1).

Histamine and LTC4 or LTB4 release. Histamine release from BAL cells showed differences between the patients with atopic and nonatopic asthma. In atopic

Table 1  Characteristics of patients with and without steroid dependent intractable asthma (SDIA) in relation to atopic and nonatopic types

<table>
<thead>
<tr>
<th>Asthma type</th>
<th>SDIA</th>
<th>No of patients</th>
<th>Age (years)</th>
<th>Serum IgE* (IU/ml)</th>
<th>IgE·Ab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopy</td>
<td>+</td>
<td>14</td>
<td>48.9</td>
<td>737 ± 463</td>
<td>10(71.4%)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>24</td>
<td>47.2</td>
<td>1151 ± 1051</td>
<td>16(66.7%)</td>
</tr>
<tr>
<td>Non-atopy</td>
<td>+</td>
<td>17</td>
<td>58.8</td>
<td>66 ± 32</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>14</td>
<td>62.7</td>
<td>49 ± 33</td>
<td>-</td>
</tr>
</tbody>
</table>

IgE·Ab: IgE antibodies for inhalant allergens.
*Mean ± SD

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subjects with SDIA, histamine release was significantly lower than in non-SDIA patients (0% vs. 16%) (p < 0.05). Histamine release in atopic non-SDIA patients was significantly higher than in nonatopic SDIA patients (p < 0.05) or nonatopic non-SDIA patients (p < 0.05). Very few of the nonatopic SDIA or non-SDIA patients showed a high level of histamine release (Fig. 2).

The release of LTC$_4$ from BAL cells induced by Ca ionophore A23187 varied from 0 to 16.8 ng/10$^6$ cells. The LTC$_4$ release was significantly lower in atopic SDIA patients (1.0 ng/10$^6$ cells) than in atopic non-SDIA patients (14.5 ng/10$^6$ cells) (p < 0.02). The LTC$_4$ release in atopic SDIA patients was also significantly lower than in nonatopic SDIA patients (5.9 ng/10$^6$ cells, p < 0.01) and nonatopic non-SDIA (4.1 ng/10$^6$ cells, p < 0.05). The LTC$_4$ release in nonatopic subjects did not differ between SDIA and non-SDIA patients (Fig. 3).

The LT$_B_4$ release in atopic subjects did not differ between SDIA patients (16.3 ng/10$^6$ cells) and non-SDIA patients (19.0 ng/10$^6$ cells). Among the nonatopic subjects, the LT$_B_4$ release in SDIA patients (36.5 ng/10$^6$ cells) was higher than that in non-SDIA patients (28.4 ng/
$10^6$ cells), although the difference was not significant. LTB$_4$ release did not differ between patients with atopic and nonatopic asthma (Fig. 4).

**BAL cell components.** The proportions of lymphocytes, neutrophils and eosinophils in BAL fluid were compared between SDIA and non-SDIA patients. The proportion of lymphocytes in the BAL fluid of atopic subjects was significantly lower in SDIA patients (8.2 %) than non-SDIA patients (16.5 %) ($p < 0.05$). The proportion of BAL lymphocytes in atopic SDIA patients was also significantly lower than that in nonatopic non-SDIA patients (21.9 %) ($p < 0.01$). However, there was no
significant difference in the proportion of BAL lymphocytes between atopic and nonatopic SDIA patients. In nonatopic subjects, the proportion of BAL lymphocytes was not also significantly different between SDIA and non-SDIA patients (Fig. 5).

For both atopic and nonatopic subjects, the proportion of BAL neutrophils was higher in SDIA patients than in non-SDIA patients, but there was no significant difference between the two groups (Fig. 6). The proportion of BAL eosinophils did not differ between SDIA and non-SDIA patients in both the atopic and nonatopic groups of subjects (Fig. 7).

**Bronchial reactivity.** Bronchial reactivity to methacholine was compared between SDIA and non-SDIA patients. Among the atopic subjects, non-SDIA patients tended to have a higher sensitivity to methacholine than SDIA patients: 6 out of 16 (37.5%) non-SDIA patients reacted to methacholine at a concentration of < 195 μg/ml, while only 1 out of 9 (11.1%) SDIA patients reacted to the same concentration (Fig. 8). In contrast, there was no difference between SDIA and non-SDIA patients among the nonatopic subjects.

**Discussion**

It is well known that IgE-mediated allergic reaction is one of the major factors contributing to the development of bronchial asthma (1-3). This allergic response is triggered by the release of chemical mediators like histamine (1-3) and leukotrienes (4, 5) from tissue mast cells, which is followed by the infiltration of inflammatory cells such as lymphocytes, neutrophils, eosinophils and basophils (6-21). The number of lymphocytes and eosinophils in BAL fluid is reported to be increased in patients with asthma when compared with the healthy controls (8, 9, 11-14, 19). An increase of BAL neutrophils has also been observed in some asthmatic patients (26), but the significance of this change is not clear. Airway inflammation has also been investigated by analyzing the release of eosinophil cationic protein, hyaluronic acid, and myeloperoxidase from inflammatory cells (27). At present, airway inflammation is considered to be an important factors in asthma, since it increases the severity of the disease (28).

In the present study, the effects of long-term glucocorticoid therapy in the inflammatory process in the airways were examined in patients with atopic and nonatopic asthma. In the atopic asthma group, the release of histamine and LTC₄ from BAL cells was significantly lower in SDIA patients than in non-SDIA patients. These results indicate that histamine and LTC₄ release from the BAL cells of atopic subjects was suppressed by long-term glucocorticoid therapy. LTB₄ release from BAL cells did not differ between SDIA and non-SDIA patients in both the atopic and nonatopic groups, indicating that LTB₄ release is little affected by long-term glucocorticoids.

Lymphocytes are increased in the BAL fluid of asthma patients in comparison with healthy subjects (9, 11, 14), and this has been shown to be due to an increase of T cell subsets (14). The median percentage of lymphocytes in asthma patients was found to be 14% by Kelly et al. (14), while that of healthy subjects was 8%, and lymphocytes > 14% was evaluated as abnormal in healthy subjects (29). An increase of BAL lymphocytes was found in many of our non-SDIA patients. The proportion of BAL lymphocytes in atopic SDIA subjects was significantly lower than in atopic non-SDIA subjects. These results demonstrate that BAL lymphocytes are suppressed by long-term glucocorticoids in atopic asthmatics.

The proportion of BAL neutrophils was increased in some of the SDIA patients from both the atopic and nonatopic groups, suggesting that long-term glucocorticoids may induce an increase of neutrophils in the BAL fluid.

To clarify the relationship between these effects of glucocorticoids and bronchial hyperreactivity, the bronchial response to methacholine was examined. The bronchi of atopic SDIA patients seemed to be less sensitive to methacholine challenge than the bronchi of atopic non-SDIA patients, but there was no difference between SDIA and non-SDIA patients in the nonatopic group.

These results demonstrated that long-term glucocorticoids suppress the release of histamine and LTC₄ from BAL cells and reduce BAL lymphocytes in patients with atopic asthma, and that such treatment may increase BAL neutrophils in both atopic and nonatopic subjects. We conclude from the study findings that long-term glucocorticoid therapy affects humoral and cellular events in the airways of atopic asthmatics more than in those of nonatopic asthmatics.
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