Specific immunotherapy downregulates peripheral blood CD4 and CD8 T-lymphocyte activation in grass pollen-sensitive asthma

M. Majori*, S. Bertacco*, M.L. Piccoli*, R. Melej*, V. Pileggi**, A. Pesci*


ABSTRACT: Several lines of evidence indicate that specific immunotherapy may act by modifying the immune responses of T-lymphocytes to the antigen.

To evaluate the effect of specific immunotherapy on the activation of T-lymphocytes by cluster of differentiation cells (CD4+ and CD8+) in peripheral blood, the expression of two surface activation markers, the p55 interleukin-2 receptor (CD25) and human leucocyte antigen (HLA)-DR, was studied prospectively on circulating CD4+ and CD8+ T-cell subsets in subjects with grass-pollen sensitive asthma before and after 1 yr of treatment with specific immunotherapy. Twenty five asthmatic patients with pollen sensitivity other than grass, out of their pollen season, served as the control group.

Specific immunotherapy improved clinical indices of disease activity including symptom scores and medication use during the pollen season of the treatment year. It had a marked effect in reducing the expression of the two activation markers, CD25 and HLA-DR, in both CD4+ (p=0.002 and p=0.005, respectively) and CD8+ (p=0.01 and p=0.01, respectively) T-cell subsets, in parallel with a significant decrease in CD23 expression on B-cells (p=0.008) and in grass-specific immunoglobulin E levels (p=0.01) in the peripheral blood of subjects with grass pollen-sensitive asthma. The decreased T-lymphocyte activation observed in immunotherapy-treated subjects after the treatment year was significant (p=0.05) in comparison with the control group.

These data add to the view that the efficacy of specific immunotherapy may be attributed to the downregulation of T-cell responses.


Although specific immunotherapy (SIT) has been used for the treatment of allergic bronchial asthma since 1911, its exact mechanism has not yet been completely clarified [1].

Several immunological changes occur during SIT that may contribute to its efficacy. These include an increase in allergen-specific immunoglobulin (IgE) antibodies [2] and moderate impairment in the synthesis of allergen-specific IgE [3], development of anti-idiotypic antibodies [4], reduced basophil reactivity and sensitivity to allergens [2], reduced allergen-stimulated lymphocyte proliferation and lymphokine production [5, 6], and the generation of allergen-specific suppressor T-cells [7].

Recently, it has been suggested that SIT alters the balance of cytokines released from helper T-lymphocytes in the respiratory tract, with a shift from the type 2 helper T (Th2)-cells, which release interleukin (IL)-4 and IL-5 in association with allergic inflammation, towards type 1 helper T (Th1)-cells that release interferon-γ which inhibits Th2 cells [8, 9].

There is increasing evidence that the bronchial inflammation characteristic of asthma represents a specialized form of cell-mediated immunity in which lymphokine products of the Th2 cells orchestrate the accumulation and activation of granulocytes. Of these, eosinophils are particularly implicated in causing the damage to the bronchial mucosa that is thought to underlie the clinical manifestation of asthma [10].

Although T-cell attraction and activation in the bronchial compartment is most evident in asthma, signs of activation of T-cells in the peripheral blood can also be measured. In acute severe [11] and moderate-to-severe [12] asthma, T-lymphocyte activation in peripheral blood has been shown to relate to the clinical expression of the disease, as reflected by the increase of cluster of differentiation (CD4+) T-lymphocytes activated in peripheral blood, and the ability to reduce this with parenteral or oral corticosteroids, respectively, in parallel with clinical improvement.

Little information is available on the possible changes following SIT in T-lymphocytes activation in peripheral blood from asthmatic subjects [13, 14].

The aim of the present study was to evaluate this effect on CD4+ and CD8+ T-cell subsets. For this purpose we examined the expression of two surface activation markers, the α-chain (Tac) of the IL-2 receptor (CD25) and the class II major histocompatibility complex antigen human leucocyte antigen (HLA)-DR, on circulating CD4+ and CD8+ T-cell subsets from subjects with grass pollen-sensitive asthma before and after 1 yr of SIT. The control group comprised 25 asthmatic patients with pollen sensitivity other than grass studied out of their pollen season.
Materials and methods

Study subjects

A group of 12 subjects with grass pollen-sensitive asthma (eight males and four females, aged 25.4±1.65 yrs (mean±SEM) range 19–37 yrs) living in the Parma area were enrolled in this study. Characteristics of the immunotherapy-treated patients are shown in table 1. As a control group, 25 asthmatic subjects with pollen sensitivity other than grass (11 Betulaceae, six Corylaceae, six Urticaceae and two Compositae) (13 males and 12 females, mean age 31.5±1.7 yrs, range 21–40 yrs) and disease of comparable severity (when compared with immunotherapy-treated patients before they had received treatment) were recruited from the outpatient clinic of the Rasori Hospital, Parma.

All patients fulfilled the criteria of the American Thoracic Society [15] for the diagnosis of asthma, and they all had clinical histories of bronchospasm after pollen exposure. The diagnosis was confirmed by a positive skin test with pollen extracts, total serum IgE levels >100 U·mL⁻¹ and the presence of allergen-specific IgE, scored from 0 to 4 (DHS CLA Allergy Test; Bayer, Milan, Italy). No patient had perennial allergy.

Patients requiring theophylline, anticholinergic, inhaled or oral corticosteroids, long-acting inhaled bronchodilators, oral bronchodilators and antihistamines were excluded from the study. Respiratory symptoms during the pollen season in immunotherapy-treated patients were controlled with inhaled short-acting bronchodilators (salbutamol) on a continuous basis or on demand.

Patients presenting with at least one of the following conditions: chronic asthma, asthma requiring one or more hospitalizations in the previous year, current smoking, previous treatment with SIT, systemic illness or pregnancy, were also excluded. Each subject gave informed and signed consent to participate in the study.

Study design

After selection, all subjects with grass pollen-sensitive asthma were monitored during the 1993 pollen season (P0) (from April 15–May 31) before the beginning of SIT.

Patients were asked to keep a diary in which they recorded: 1) symptoms of asthma according to a 0–3 grading (0: absent, 1: mild, 2: moderate, 3: severe); 2) number of puffs (100 µg) per day of salbutamol required to control symptoms; and 3) peak expiratory flow (PEF) as the best of three pretreatment measurements taken twice daily using a mini-Wright portable peak flow meter (Markos, Italy). From October 1993 to October 1994 each subject was submitted to SIT with an aluminium hydroxide-absorbed grass pollen extract (Bayer, Milan Italy). The dose scheduled was administered subcutaneously and was similar in all subjects. The initial dose was 2.5 activity units (AU) of alpha fraction (200 AU = skin activity reference allergen/histamine 1% (SARAH)), and it was first increased weekly up to a maintenance dose of 800 AU. The maintenance dose was reached after approximately 3 months, and then this dose was administered every 2 weeks for 1 month and thereafter every 4 weeks. During the pollen sea-son, the dosages were lowered to 40–60% of the maximum amount reached. Patients underwent pulmonary function testing, i.e. the forced expiratory volume in one second (FEV1) and methacholine sensitivity (provocative concentration of methacholine causing a 20% fall in FEV1) (Spiroflow; Morgan, Kent, UK), and a peripheral venous blood sample was taken for lymphocyte subset analyses and measurements of grass-specific IgE levels before the beginning of SIT (T0, October 1993) and following 1 yr of treatment (T1, October 1994). During the 1994 pollen season (P1) all patients were monitored for symptoms, salbutamol consumption and PEF, according to the criteria already given for P0. Grass pollen counts for the two pollen seasons were obtained from Agenzia Regionale per la prevenzione e l’ambiente dell’Emilia, Romagna, Sezione Provinciale di Parma.

The control group was sampled once only, out of their pollen season.

Analysis of lymphocytes

Specific binding of monoclonal antibodies (mAB) was analysed by direct immunofluorescence according to standard methods recommended by Becton-Dickinson Monoclonal Center (Mountain View, CA, USA) using a flow cytometer.
cytometer (FACSScan, Becton-Dickinson). In brief, 100 µL of heparinized whole blood was incubated in the presence of saturating concentrations of fluorescein- or phycoerythrin-conjugated mAB at room temperature for 20 min. Erythrocytes were lysed by adding 2 mL lysis solution for 10 min. Cells were washed twice with phosphate-buffered saline containing 2% fetal calf serum and 0.1% sodium azide. Cytofluorimetric analysis was performed by imposing an electronic gate on forward-scatter and side-scatter dot plots for peripheral blood lymphocytes. The number of immunofluorescence-positive cells was determined in 10,000 analysed cells. Specific binding of mAB was controlled by subtraction of isotype-matched mouse immunoglobulins. mAB against CD3 (T-cells), CD4 (T-helper-inducer), CD8 (T-suppressor-cytotoxic), CD19 (B-cells), CD25 (p55 IL-2 receptor), HLA-DR and CD23 (B-cells expressing the low-affinity Fc IgE receptor) were purchased from Becton-Dickinson.

Simultaneous T-cell subset analyses (CD4 and CD8) and activation studies (expression of activation markers CD25 and HLA-DR) were performed on peripheral blood lymphocytes.

Data analysis

Results were expressed as medians and range, or means±SEM when appropriate. In immunotherapy-treated patients, data before SIT were compared with results after SIT using Wilcoxon’s signed rank test. The Mann-Whitney U-test was used to compare data between immununotherapy-treated patients and the control group. Correlations were examined by Spearman’s rank correlation coefficient. A p-value of <0.05 was considered significant.

Results

All subjects had mild symptomatic asthma (cough and/or dyspnoea) only during the pollen season. The comparison of symptom scores and salbutamol consumption during P0 and P1 showed a statistically significant decrease for both symptom scores (from 4.3±0.5 to 3.1±0.4, p=0.05) and salbutamol consumption (from 5.6±0.9 to 2.5±1.9, p<0.05). PEF data were not analysed because they were only available for seven patients.

Grass pollen counts in Parma were similar during the same periods (fig. 1). Grass-specific IgE levels were significantly reduced (from 3.75±0.13 to 3±0.4, p=0.01) where-as there were no significant differences in the FEV1 (from 99.5±3.3 to 97.2±2% of predicted value, ns) and methacholine sensitivity (from 4.99±0.87 to 5.4±1 mg·mL⁻¹, NS) at T1 when compared to T0.

Flow cytometric analysis of peripheral blood lymphocytes at T0 and T1 showed no significant differences in the percentage of T-lymphocytes (CD3+) or B-lymphocytes (CD19+), nor was there any change in either the CD4+ or CD8+ T-cell subsets (table 2). However, a comparison of the two activation markers CD25 and HLA-DR showed significant reduction at T1 in both CD4+ (p=0.002) and CD8+ (p=0.005) T-cells (fig. 2).

Fig. 2. Percentages of peripheral blood CD4+ cells expressing the two activation markers, p55 interleukin-2 receptor (CD25) and human leukocyte antigen (HLA)-DR, from subjects with grass pollen-sensitive asthma before (T0) and after (T1) specific immunotherapy. Horizontal bars represent group mean values.

Fig. 3. Percentages of peripheral blood CD8+ cells expressing the two activation markers, p55 interleukin-2 receptor (CD25) and human leukocyte antigen (HLA)-DR, from subjects with grass pollen-sensitive asthma before (T0) and after (T1) specific immunotherapy. Horizontal bars represent group mean values.
Table 2. – Lymphocyte subpopulations from immunotherapy-treated patients and control group

<table>
<thead>
<tr>
<th></th>
<th>CD3</th>
<th>CD4</th>
<th>CD8</th>
<th>CD19</th>
<th>CD23</th>
<th>CD4/CD25</th>
<th>CD4/HLA-DR</th>
<th>CD8/CD25</th>
<th>CD8/HLA-DR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before SIT (A)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>73.4</td>
<td>38.1</td>
<td>34.5</td>
<td>12.3</td>
<td>9.3</td>
<td>2.9</td>
<td>1.95</td>
<td>0.2</td>
<td>2.85</td>
</tr>
<tr>
<td>Range</td>
<td>70.1–82.6</td>
<td>30.2–55.6</td>
<td>22.2–46.9</td>
<td>5.5–22.1</td>
<td>3.6–16.2</td>
<td>1.9–4.5</td>
<td>1.2–3.0</td>
<td>0–1.3</td>
<td>0.5–8.6</td>
</tr>
<tr>
<td>After SIT (B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>73.2</td>
<td>35.9</td>
<td>37.2</td>
<td>9.6</td>
<td>7.1</td>
<td>1.0</td>
<td>1.75</td>
<td>0.1</td>
<td>2.2</td>
</tr>
<tr>
<td>Range</td>
<td>59.9–79.4</td>
<td>30.7–55.5</td>
<td>22.4–46.0</td>
<td>4.6–14.6</td>
<td>3.6–10.8</td>
<td>0.6–1.8</td>
<td>0.7–2.5</td>
<td>0–0.9</td>
<td>0.6–3.8</td>
</tr>
<tr>
<td>Controls (C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>72.2</td>
<td>42.0</td>
<td>32.1</td>
<td>9.6</td>
<td>8.6</td>
<td>2.8</td>
<td>1.9</td>
<td>0.2</td>
<td>2.9</td>
</tr>
<tr>
<td>Range</td>
<td>53.3–87.8</td>
<td>27.4–67.0</td>
<td>20.5–55.9</td>
<td>2.9–19.9</td>
<td>2.0–15.4</td>
<td>0.7–4.8</td>
<td>1.2–3.7</td>
<td>0–1.7</td>
<td>1.3–7.0</td>
</tr>
<tr>
<td>A versus B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p=0.008</td>
<td>p=0.002</td>
<td>p=0.005</td>
</tr>
<tr>
<td>A versus C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B versus C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p=0.05</td>
<td>p=0.001</td>
<td>p=0.01</td>
</tr>
</tbody>
</table>

SIT: specific immunotherapy; NS: nonsignificant.

Discussion

This study showed a downregulation of CD4 and CD8 T-lymphocyte activation in parallel with a significant reduction in CD23+ B-cells and grass-specific IgE levels in the peripheral blood of subjects with grass pollen-sensitive asthma after 1 yr of SIT. Moreover, the reduction in symptoms and need for drugs during the pollen season of the treatment year confirmed the clinical efficacy of SIT in grass pollen-sensitive asthma [16, 17]. Since this study examined peripheral blood T-cells in patients out of the pollen season, when they were clinically healthy subjects, it can only be presumed that the downregulation of T-lymphocyte activation in peripheral blood correlates with the clinical success of SIT, and it remains to be studied whether nonresponders to SIT lack similar changes in immunoreactivity to the injected allergen. Moreover, studying peripheral blood T-cells during the pollen season limits the reliability of the study because it has been demonstrated that exposure to artificial or natural antigens alters T-cell subsets [18, 19] or activation [20] in peripheral blood from allergic asthmatic subjects.

To the best of our knowledge, there have been no studies on the effect of SIT on T-lymphocyte activation in peripheral blood from subjects with grass pollen-sensitive asthma.

CD25 and HLA-DR are well-established markers of T-lymphocyte activation. The fact that SIT of the asthmatic patients was associated with a reduction in the percentages of peripheral blood CD4+ and CD8+ T-lymphocytes expressing these markers, but not a reduction in the overall numbers of these cells, suggests that SIT acts directly on these cells to reverse their activated status.

The production of allergen-specific IgE is under the control of activated T-cells through the release of cytokines such as IL-4, which stimulates B-cells to proliferate and to undergo isotype switching to produce IgE [21]. It can be speculated, therefore, that the decrease in CD23 expression on B-cells and in grass-specific IgE levels in the peripheral blood of asthmatic subjects after SIT may be a consequence of the downregulation of T-lymphocyte activation in the same compartment. The downregulation of CD8 (other than CD4) T-lymphocyte activation may be important in view of the increasing evidence for the existence of Th2-like CD8+ T-cells [22–26]. It is now clear that CD8+ cells may also secrete cytokines such as IL-4 and IL-5, which are essential co-factors in IgE synthesis and the accumulation and activation of eosinophils, respectively [27, 28]. In this regard, Walker et al. [29] demonstrated an upregulation of CD8 T-lymphocyte activation in bronchoalveolar lavage and peripheral blood of nonallergic asthmatics, and Hämelsmann et al. [30] demonstrated a critical role for CD8+ T-cells in the development of airway hyperresponsiveness in a murine model of airway sensitization.

In conclusion, this study shows a downregulation of peripheral lymphocyte activation in both CD4+ and CD8+ T-cell subsets in parallel with a significant decrease in CD23 expression on B-cells and in grass-specific immunoglobulin levels in subjects with grass pollen-sensitive asthma after 1 yr of specific immunotherapy. These data add to view that the efficacy of specific immunotherapy may be attributed to the downregulation of T-cell responses.

Acknowledgements: The authors would like to thank E. de Young for help with the final text of this paper, I. Spanevello for expert technical assistance, F. Cassone for grass pollen counts and F. Gardini for statistical analysis.

References


