Decreased Production of CD8 (T8) Antigen After Immunotherapy

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The working mechanism(s) of immunotherapy still remains ill defined. As T cells bearing CD8 antigen possess suppressor/cytotoxic function, this study was conducted to examine the effect of immunotherapy on the production of CD8 antigen. Peripheral blood mononuclear cells (MNC) were obtained from 21 newly diagnosed and 23 hyposensitized (>1 year) asthmatic children and 13 age-matched normal children. MNC were stimulated with crude mite extract (Dermatophagoides farinae) for 7 days and with phytohemagglutinin and concanavalin A for 3 days. The CD8 antigen and interleukin-2 receptor (IL-2R) in plasmas and culture supernatants were measured by CELLFREE T8 and IL-2R test kits (T Cell Sciences, USA). The results showed the following. (1) Plasma CD8 antigen was markedly increased in new patients compared to normals (536.7 ± 212.3 vs 222.5 ± 104.0 units/ml; P < 0.001) and decreased to normal after immunotherapy (275.7 ± 98.5 units/ml). (2) When stimulated with mite allergen, MNC from both new and hyposensitized patients produced a much greater amount of CD8 antigen compared to those from normals. However, after immunotherapy MNC tended to produce less CD8 antigen, although not to a significant degree. (3) No difference in CD8 antigen production was seen among three groups when lymphocytes were stimulated with mitogens. (4) Production of CD8 antigen paralleled that of IL-2R. Thus, CD8 production was specifically decreased after immunotherapy and this fact reflects a hyposensitized state of T cells after long-term, repeated injection of allergens.

KEY WORDS: CD8 antigen; immunotherapy.

INTRODUCTION

Patients with allergic diseases are characterized by the presence of elevated total serum IgE and specific IgE antibodies against a wide variety of environmental allergens (1–3) and enhanced allergen-induced lymphoproliferation (4–6). As IgE production is a highly T cell-dependent process (7), and increased IgE production has been observed frequently in patients with T-cell deficiency (8, 9), the balance between helper and suppressor T-cell function has been studied extensively in atopic patients.

Enumeration of T-cell subsets has become a widely used practice to evaluate immune status, especially after the availability of monoclonal antibodies against T-cell surface molecules (10). T cells bearing CD8 (T8) antigen have been shown to possess suppressor/cytotoxic function (10). Conflicting data have been reported regarding the number of CD8 + T cells in allergic patients. Leung et al. (11) reported decreased CD8 + cells in patients with atopic dermatitis and Engel et al. (12), Kus et al. (13), and Bruijnzeel et al. (14) reported similar results in patients with bronchial asthma. However, our studies (6, 15) and studies by Lison et al. (16) and Schuyler et al. (17) found normal CD8 + cells in asthmatic patients.

Recently, Fujimoto et al. (18) reported spontaneous release of CD8 molecules from human T cells. By using two monoclonal antibodies recognizing different epitopes of human CD8 antigen, Brown et al. (19) developed a sandwich enzyme-linked immunoabsorbent assay which was able to quantitate cell-free human CD8 antigen. As immunotherapy (hyposensitization) has been found to generate suppressor-cell activity for both allergen-induced lymphoproliferation (4–6, 20) and IgE production (21), the study of changes of CD8 antigen production and/or secretion after immunotherapy may provide some insight into the working mechanisms of this kind of treatment. Moreover, there is still no such study in the literature.
Table I. Characteristics of Study Populations

<table>
<thead>
<tr>
<th>Subject</th>
<th>No. of cases</th>
<th>Sex</th>
<th>Mean age (years)</th>
<th>Total serum IgE (mean ± SD, IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normals</td>
<td>13</td>
<td>8 M</td>
<td>12.1</td>
<td>123 ± 51</td>
</tr>
<tr>
<td>New patients</td>
<td>21</td>
<td>13 F</td>
<td>11.7</td>
<td>1185 ± 443*</td>
</tr>
<tr>
<td>Hyposensitized</td>
<td>23</td>
<td>14 F</td>
<td>11.4</td>
<td>1526 ± 555*</td>
</tr>
</tbody>
</table>

*Patients who were freshly diagnosed and had never received immunotherapy before.

*M < 0.01.

MATERIALS AND METHODS

Study Populations. The characteristics of the study populations are shown in Table I. They were comparable with regard to sex and age, but the patients had much higher total serum IgE than did the normals. All patients were sensitive only to house dust mite (*Dermatophagoides farinae*) in terms of a positive history and positive skin test and a 2+ or greater RAST, whereas the normal subjects showed a negative skin test and RAST. Environmental control was taught, immunotherapy was started with weekly injections, and the antigen dose was increased as rapidly as possible until a maximal tolerated dose was reached (usually within 6 months). This dose was then maintained at 4-week intervals. The treated patients had been receiving immunotherapy with crude mite extract for 1 to 2 years, with a mean of 1.4 years, and all patients were considered to be good responders as judged by the decreased frequency and shortened duration of attacks and diminished requirement for medications. No steroids had been given for at least 2 weeks and bronchodilators were withheld for at least 6 hr before blood samplings. Blood from hyposensitized patients was drawn for study just before allergen shots.

Preparation of Crude Mite Allergen. Crude extract of the house dust mite (*D. farinae*) was generously supplied by Torii & Co., Tokyo. The mite allergen was extracted according to the method of Haida et al. (22). The protein content of the extract was determined by Bio-Rad protein assay kit (Richmond, CA). The same extract was used for skin testing, immunotherapy, and in vitro culture experiments.

Preparation of patients' Plasma and Culture Supernatants. Heparinized whole blood was drawn from each subject between 2 and 4 PM and at least one normal subject was included in each experiment. After centrifugation, plasma was collected and stored at −70°C. Peripheral blood mononuclear cells (MNC) were isolated by Ficoll/Hypaque density-gradient centrifugation (23). After washing with RPMI-1640 (GIBCO) three times, MNC were adjusted to a concentration of $2 \times 10^6$ cells/ml in complete culture medium (RPMI-1640 supplemented with 10% heat-inactivated fetal calf serum, 100 units penicillin/ml, 100 μg streptomycin/ml, and 2 mM L-glutamine/ml) containing no or varying concentrations of concanavalin A (Con A) (0–80 μg/ml), phytohemagglutinin (PHA) (0–2%), and crude mite extract (0.1, 1, 10, 100 μg/ml). Cultures were done in a humidified, 37°C, 5% CO₂ incubator and culture supernatants (containing soluble CD8 antigen and interleukin-2 receptor, SIL-2R) were collected on days 0, 1, 2, 3, and 7 by centrifugation and stored at −70°C until testing.

Quantitation of CD8 Concentrations in Culture Supernatants and Plasmas. These were determined using a CELLFREE T8 test kit (T Cell Sciences, Cambridge, MA). The principle of the method was a sandwich enzyme immunoassay based on the report of Brown et al. (19), using two murine anti-CD8 monoclonal antibodies (mAb) recognizing different epitopes of CD8 molecule. The first anti-CD8 mAb was absorbed onto a polystyrene 96-well microtiter plate. CD8 present in the samples or standards bound to the antibody-coated wells; unreacted sample components were removed by washing. The horseradish peroxidase (HRP)-conjugated second anti-CD8 mAb was then added, which bound to the CD8 captured by the first antibody and completed the sandwich. After the removal of unbound HRP-conjugated anti-CD8 by washing, O-phenylenediamine (OPD) was added to the wells. A colored product was formed in proportion to the amount of CD8 present in the sample. The reaction was terminated with stop solution (2 N H₂SO₄) and absorbance at 490 nm was measured. A standard curve was constructed using standards of a known concentration supplied by the manufacturer, and unknown samples were determined from the standard curve. The lower limit of the test kit was about 60 U/ml. Spontaneous production of CD8 antigen in unstimulated culture supernatants increased gradually and peaked at the sixth day of cultivation, with an average of 100 U/ml for normals, 158 U/ml for new patients, and 130 U/ml for treated patients. The net CD8 productions were calculated by subtracting the CD8 concentrations of unstimulated cultures from the values of stimulated counterparts.