Lymphocyte Apoptosis: Mechanism of Specific Immunotherapy of Atopic Diseases

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Effect of specific immunotherapy on the expression of activation markers, apoptosis marker CD95, and peripheral blood lymphocyte morphology were studied in patients with atopic bronchial asthma. Specific therapy activated T and B cells and triggered lymphocyte apoptosis. Apoptosis may play an important role in induction of allergen tolerance during specific therapy.

Key Words: *lymphocytes; apoptosis; specific immunotherapy*

Today specific immunotherapy (SIT) is virtually the sole method suppressing for a long time the excessive production of immunoglobulin E (IgE) in patients with atopic bronchial asthma [1,2]. Although SIT was proposed for treating hay fever in 1911, the mechanisms of its therapeutic action are still obscure. The role of blocking antibodies, activation of suppressor lymphocytes, induction of anti-idiotypical antibodies to IgE, etc., are discussed [7]. From experimental and clinical findings it can be concluded that SIT triggers several mechanisms of immunological tolerance [3]. There are indirect signs of triggering of the mechanisms of B cell clonal anergy and terminal differentiation of B cells under the effect of SIT. A possible role of SIT in the development of the major mechanism of immunological tolerance, clonal deletion, is still unknown. Clonal deletion is realized by triggering the mechanism of programmed cell death — apoptosis — in corresponding cells [4]. Lymphocyte apoptosis is preceded by surface expression of protein denoted as CD95 antigen (Fas-antigen), a receptor for a still little known ligand triggering apoptosis [6,8]. Apoptosis is a result of imbalance between cell activation mechanisms primarily discoordinated accumulation of cytoplasmic Ca^{2+} and increased activity of protein kinase C [4]. SIT is associated with activation of IgE-producing lymphocytes (increased level of serum IgE and increased production of IgE *in vitro*) [3].

Our purpose was to examine the effect of SIT on the count of peripheral blood lymphocytes which express activation or apoptosis markers (CD95) and morphological signs of apoptosis.

MATERIALS AND METHODS

The study was carried out on peripheral blood lymphocytes of 12 patients with atopic bronchial asthma during remission, administered an accelerated SIT course at the Institute of Immunology, Ministry of Health of Russia. Surface markers and blood cell morphology were studied before and immediately after SIT.

Mononuclear cells were isolated from venous blood by centrifugation on a Ficoll-Verografin density gradient. Cells expressing membrane antigens were counted using indirect immunofluorescence with murine monoclonal antibodies and FaB fragments of goat immunoglobulins to mouse antibodies conjugated with fluorescein isothiocyanate. The preparation was examined under a light microscope in a water immersion medium. The main populations and subpopulations of lymphocytes were assessed: $CD3^+$ (T lymphocytes), $CD4^+$ (helper-inducer T cells), $CD8^+$ (suppressor cytotoxic T lymphocytes), $CD72^+$ (B lymphocytes); lymphocytes with early

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signs of activation: CD25⁺ (interleukin-2 receptor), CD71⁺ (transferrin receptor), and CD23⁺ (low-affinity receptor for IgE Fc-fragment, marker of B lymphocyte activation). Lymphocytes with late signs of activation were counted: HLA-DR⁺-cells and cells "ready" to apoptosis (by expression of CD95). The specificity of fluorescence was analyzed in at least 200 cells [5]. Cells with the monocyte morphology were disre-garded. Cell viability control with Trypan Blue (at least 98%) and nonspecific binding of labeled serum (no more than 4%) were performed in each test.

Blood smears were routinely prepared, fixed with methanol, and stained by the Romanovskii-Giemsa method. Differential blood count was determined in at least 200 leukocytes. Lymphocytes in apoptosis were characterized by nuclear fragmentation. Results were statistically processed using Student's *t* test.

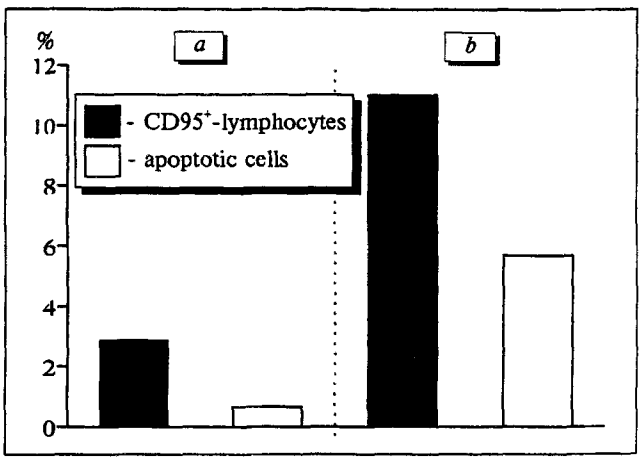

Fig. 1. Changed number of lymphocytes expressing CD95 antigen or morphological signs of apoptosis before (*a*) and after specific immunotherapy (*b*).

RESULTS

Study of SIT effect on the expression of surface markers characterizing main lymphocyte populations and subpopulations showed no essential changes in the counts of T and B cells (Table 1).

There were no significant changes in the regulatory lymphocyte subpopulations, although there was a tendency toward an increase in the number of CD8⁺ cells among peripheral blood lymphocytes.

SIT effect on activation markers of lymphocytes was quite different (Table 2).

The treatment promoted a marked increase in the number of lymphocytes carrying the "early" activation markers (CD25⁺ and CD71⁺) and of lymphocytes expressing the "late" activation marker (DR⁺ lymphocytes). This was paralleled by a more than 2-fold increase in the count of activated B cells (CD23⁺). These changes in antigenic characteristics of lymphocytes in patients with atopic asthma caused by SIT indicate marked activation of both T and B cells under the effect of specific allergen. These results account for the marked increase in IgE production after a short-term SIT [3].

However, these results do not explain the mechanism of therapeutic effect of SIT. Therefore, we analyzed changes in CD95⁺ lymphocytes. Since CD95 is a receptor whose stimulation triggers apoptosis, an almost 4-fold increase in the number of CD95⁺ lymphocytes ($p<0.0001$) can be regarded as a mechanism promoting the triggering of apoptosis of cells specific to this antigen (Fig. 1). In order to verify this hypothesis, we counted peripheral blood lymphocytes with morphological signs of apoptosis.

Figure 1 shows that SIT promoted an increase in the number of lymphocytes expressing CD95 antigen and in the number of cells "entering apoptosis" ($p<0.01$).

Therefore, SIT induces ambivalent changes in human immune system: activation of immune system

Table 1. Effect of SIT on Population and Subpopulation Composition of Peripheral Blood Lymphocytes in Patients with Atopic Asthma (*M±m*, *n*=12)

Examined	CD3⁺	CD4⁺	CD8⁺	CD72⁺
Before SIT	68.57±2.08	33.15±2.99	27.67±3.44	10.86±0.86
After SIT	69.30±4.50	35.05±2.05	31.30±3.80	11.38±1.42

Table 2. Effect of SIT on the Number of Peripheral Blood Lymphocytes Expressing Activation Markers in Patients with Atopic Asthma (*M±m*, *n*=12)

Examined	CD23⁺	CD25⁺	CD71⁺	DR⁺
Before SIT	5.46±2.16	8.10±1.58	6.74±2.07	14.45±2.83
After SIT	12.52±1.94*	16.27±2.64*	11.52±1.39	26.11±3.35*

Note. *$p<0.05$ in comparison with examination before SIT.

leading to increased production of IgE, on the one hand, and triggering of lymphocyte apoptosis leading to suppression of reaction to antigen and of IgE production, on the other.

The relationship between the increment in the count of $CD95^+$ cells and clinical efficacy of SIT in an individual patient will be analyzed in further studies and an attampt to detect a tentative selective increase in the expression of CD95 in certain lymphocyte subpopulations (phenotyping using "double label") will be made.

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