IMMUNOFLUORESCENCE STUDIES OF IMMUNOGLOBULIN RECEPTORS OF B-LYMPHOCYTES

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Surface immunoglobulin receptors of B-lymphocytes were studied by the direct immunofluorescence method. Labeled antisera against human IgE, IgG, and globulins were used. Lymphocytes were obtained from patients with atopic hay asthma. On average 2.1 ±0.33% of lymphocytes were found to have receptors for IgE, 8.4±0.63% for IgG, and 18.7±1.16% for human globulins. The character of luminescence of the B-lymphocytes is described.

KEY WORDS: immunoglobulin receptors; B-lymphocytes; IgE; IgG.

The study of surface immunoglobulin receptors of B-lymphocytes is currently the subject of intensive research [4]. The specificity of the receptors, the classes of immunoglobulins to which they belong, and changes during ontogeny and in certain diseases have been studied [5, 11]. The question of lymphocytes carrying receptors for IgE has received the least study. Such work as has been published [10, 12] gives no grounds for any definite conclusions on this matter.

In the investigation described below an attempt was made to study surface immunoglobulin receptors of B-lymphocytes for IgE.

EXPERIMENTAL METHOD

Lymphocytes were obtained from patients with atopic hay asthma. The lymphocytes were isolated by Böyum's method [2]. Heparinized blood was layered on a ficoll-verografin gradient with a density factor of 1.077 and centrifuged for 30 min at 1500 rpm at room temperature. After centrifugation a white ring of lymphocytes was formed in the interphase and was carefully removed and washed twice with medium No. 199.

Serum against human IgE was obtained from Pharmacia (Sweden), and labeled with fluorescein isothiocyanate in the usual way. The labeled antiserum was used in the experiments in a dilution of 1:8. Labeled antiserum against IgG was obtained from Biomed (Poland) and labeled antiglobulin serum against human globulins was prepared at the N. F. Gamaleya Institute of Epidemiology and Microbiology.

To 0.2 ml of the cell suspension was added an equal volume of labeled serum, previously adsorbed on human embryonic liver powder. The cell suspensions with labeled sera were kept for 30 min at 4°C, then washed 3 times, and 1 drop of cell suspension in buffered glycerol was examined in the ML-4 luminescence microscope. In some experiments a cell monolayer on cover slips was studied after preliminary application of a formvar film. Parallel staining of the cells on the coverslip and in the suspensions yielded identical results. The number of luminescent lymphocytes, having different types of luminescence, were counted: granular at the periphery of the lymphocyte, punctate with single or multiple points of fluorescence, and lymphocytes with "caps" of fluorescence at one pole. The specificity of fluorescence was determined in accordance with the criteria suggested by Coons and Kaplan [3]. The lymphocytes were identified by a parallel phase-contrast study.

EXPERIMENTAL RESULTS

Immunoglobulin receptors of B-lymphocytes of 21 patients with atopic hay asthma, seven of whom showed increased sensitivity of grass pollen, four to tree pollen, and ten patients had increased sensitivity to both tree and grass pollen simultaneously, were studied. All the patients had positive skin tests with the correspond-

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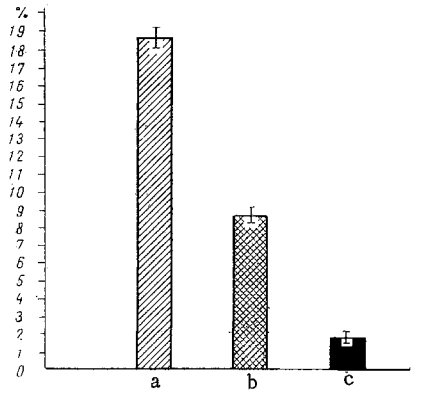

Fig. 1. Number of fluorescent lymphocytes (in %) in patients' peripheral blood; a) lymphocytes treated with antiglobulin serum; b) antiserum against IgG; 3) antiserum against IgE.

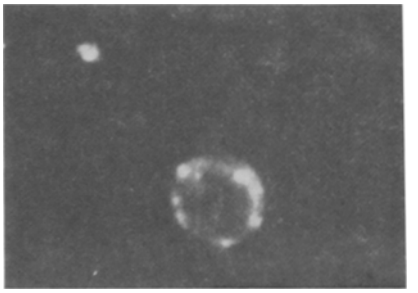

Fig. 2. Fluorescent lymphocyte after treatment with labeled antiserum against IgE, 450×.

ing allergens, prepared from tree and grass pollen. No patient received any treatment during the period of investigation, and no specific desensitizing treatment had been given to these patients previously. During the investigation no correlation was found between the duration of the disease and the number of B-lymphocytes carrying surface IgE receptors. For instance, among lymphocytes obtained from one patient with an 11-year history of the disease IgE receptors were found in 1% of cases, whereas in a patient with only a two-year history of the disease the number of B-lymphocytes carrying IgE receptors was 5%.

Fluorescent lymphocytes with IgE receptors usually had fine granular fluorescence at the periphery, there were also some cells with the punctate fluorescence, but no cells with fluorescence in the form of a cap.

Since immunoglobulins on the surface of the lymphocytes and the immunoglobulins secreted by them exhibit identical specificity, this suggests that a serum IgE level of 0.003 mg/ml, rising sharply in allergic diseases [1], can be attributed to some extent to a small increase in the number of lymphocytes with IgE receptors in the peripheral blood of patients with atopic hay asthma (Fig. 1). The fine granular fluorescence of lymphocytes with IgE receptors is evidently due to a small number of specific receptors on the surface of the B-lymphocyte (Fig. 2).

It has recently been shown that different types of receptors occur on the surface of B-lymphocytes: immunoglobulin receptors, receptors for the third component of complement, and also receptors for the Fc-fragment. The density of distribution of these receptors on the surface of the B-cells is 10^5 per cell [13]. Human immunoglobulin receptors are represented by the following classes of immunoglobulins: IgM, IgG, IgA, IgD, and IgE.

It has been shown that lymphocytes carrying IgM determinants on their membrane are precursors of cells producing immunoglobulins of different classes [9]. During ontogeny the number of lymphocytes carrying IgM on their membrane falls, but the number of lymphocytes carrying determinants of immunoglobulins of other classes (IgG [7] and IgD [14]) on their membrane increases.

Incubation of B-lymphocytes with antisera against immunoglobulins is accompanied by redistribution of the molecules on their membrane; the process of redistribution of molecules on the membrane induced by antiserum against immunoglobulins of a particular class takes place independently of other classes of immunoglobulins [6, 8]. The lymphocyte membrane is a dynamic structure and the character of its fluorescence is connected with the arrangement of the receptors on the surface of the lymphocyte. Fluorescence in the shape of a cap is due to an uneven distribution of receptors on the surface of the membrane as a result of its stimulation by the antigen and concentration of receptors at one pole of the cell.

It should be noted that when labeled antiglobulin serum was used, the fluorescent lymphocytes showed all three types of fluorescence: There were lymphocytes with granular fluorescence at the periphery of the cell, lymphocytes with single (3 or 4) and with multiple fluorescent points all over the surface of the cell, and also cells with fluorescence in the shape of a cap.

When labeled antiserum against IgG was used the lymphocytes had mainly punctate or granular fluorescence, and fluorescence in the form of a cap was extremely rare.

The experimental results showed that when patients' lymphocytes were treated with labeled antiserum against IgE, fluorescent lymphocytes were found on average in $2.1 \pm 0.33\%$ of patients, when antiserum against IgG was used, fluorescent cells were found in $8.4 \pm 0.63\%$ of patients, and when antiserum against human globulins was used they were found in $18.7 \pm 1.16\%$ of patients.

The study of immunoglobulin receptors of B-lymphocytes with the aid of labeled monospecific antisera and, in particular, antiserum against IgE in the course of specific desensitizing treatment of patients with atopic hay asthma will be the subject of future research.

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