CHANGES IN THE NUCLEAR CHROMATIN OF CIRCULATING BLOOD LYMPHOCYTES IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS DETECTED BY CYTOSPECTROFLUORIMETRY

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Phytohemagglutinin (PHA) does not increase the fluorescence of acridine orange bound with the chromatin of lymphocytes from patients with systemic lupus erythematosus, incubated for 1 h in medium containing autolytic serum and Eagle's medium, by contrast with the significant increase in luminescence of the dye produced by the action of PHA on material from healthy donors. The addition of total gamma-globulin, immune gamma-globulin, antibodies against single-stranded DNA, and albumin isolated from the serum of a patient with systemic lupus erythematosus in all cases led to a significant increase in the intensity of luminescence of the dye and absence of a reaction to the mytogen. It can be concluded that the nuclear chromatin of the lymphocytes is exposed to exogenous effects which are not specific in nature.

The method of cytospectrofluorescence enables physicochemical modifications of the principal genetic structure of the cell (chromatin) to be analyzed directly in the cell itself [6, 7]. In a previous paper [3] details were given of changes in the physicochemical structure of the nuclear chromatin of circulating blood lymphocytes of persons with certain hereditary diseases. The factor causing these changes may be either endogenous or exogenous relative to the cell investigated. Analysis of the localization of this factor is of considerable importance for it points out the direction of the search in order to identify this factor and, possibly, to remove it. Before such an analysis can be undertaken, it is necessary to determine whether, in principle, modifications can be produced in the chromatin by exogenous factors which are not typical mitogens.

The object of the present investigation was to determine the possibility of quantitative analysis of the initial stages of chromatin modification during blast transformation of lymphocytes induced by exogenous factors relative to the cells studied, and in the event of success to conduct such an analysis.

Circulating blood lymphocytes from patients with systemic lupus erythematosus were chosen as the test object, first, because in this disease there is a wide spectrum of changes which can be tested in a system exogenous relative to the cell (the blood serum) and, second, cytomorphological changes have been demonstrated in the lymphocytes of healthy donors when cultivated in serum from patients [1, 2].

EXPERIMENTAL METHOD

Cytospectrofluorometry was the principal method of investigation. The apparatus, the details of its operation, and the method of isolation and cultivation of the lymphocytes were described previously [3].

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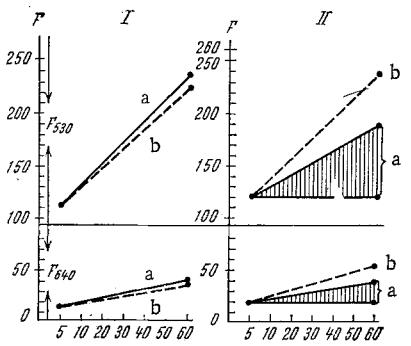

Fig. 1

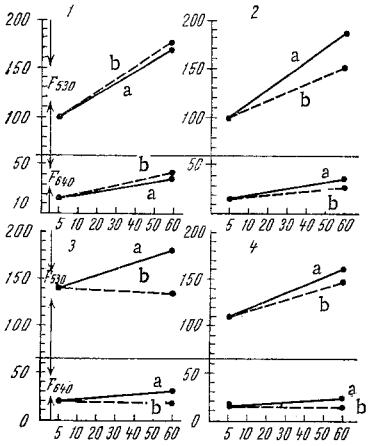

Fig. 2

Fig. 1. Fluorescence (λ 530 and 640 nm) of acridine orange bound with chromatin of human circulating blood lymphocytes versus incubation time: I) lymphocytes from patients with systemic lupus erythematosus without PHA (a) and in the presence of PHA (b); II) lymphocytes of healthy donors without PHA (a) and in the presence of PHA (b). Here and in Fig. 2: abscissa, incubation time (in min); ordinate, intensity of fluorescence.

Fig. 2. Fluorescence (λ 530 and 640 nm) of acridine orange bound with the chromatin of the circulating blood lymphocytes of healthy donors versus incubation time during exposure of various exogenous factors: 1) addition of total γ-globulin (1 mg to 5 ml); 2) addition of immune γ-globulin (1 mg to 5 ml) to culture medium; 3) addition of antibodies against single-stranded DNA isolated from serum of a patient with systemic lupus erythematosus (1 mg to 5 ml); 4) addition of serum albumin (5 mg to 5 ml); a) without PHA; b) with PHA.

Luminescence of acridine orange, bound with DNP complexes of the nuclei of the lymphocytes, was used as the test. This paper describes the results obtained by an investigation of 42 healthy donors and 28 patients with systemic lupus erythematosus.

EXPERIMENTAL RESULTS

Incubation of lymphocytes from healthy donors for 1 h led in about 40% of cases to an increase in the intensity of fluorescence by up to 40% (Fig. 1, II). These results demonstrate the high resolving power of the method, so that it is suitable for testing the extraordinary reactivity of lymphocytes, which evidently respond to the very slight disturbing effects of blood serum even from healthy donors. Meanwhile these cells react additionally to phytohemagglutinin (PHA). Under the influence of PHA their activity increased. Only in 3-5% of cases did the lymphocytes not react to PHA, a result which agrees in general with those obtained by analysis of the level of PHA synthesis in normal and PHA-stimulated cells [5]. It can accordingly be concluded from the use of the spectrocytofluorometric method that some activation of the nuclear chromatin of the lymphocytes takes place in a high proportion of cases and it is not completed by their blast transformation. This conclusion is based on results obtained by other workers who, when using cytomorphological analysis, failed to observe any substantial blast transformation of lymphocytes of healthy donors in the absence of stimulation by mytogen [4].

A picture quite different from normal was observed when lymphocytes from patients with systemic lupus erythematosus were tested. Cultivation of lymphocytes from the patients for 1 h led in 85% of cases (24 of 28 patients) to a significant ($P < 0.001$) increase in the intensity of luminescence by 40-60% in both regions of the spectrum. In addition, and this is evidently the most important discovery, the presence of

PHA in the culture medium did not cause any additional increase in the intensity of luminescence (Fig. 1, I). This suggests that in systemic lupus erythematosus the patient's lymphocytes are capable of spontaneous activation by exogenous factors present in the patient's blood serum. Whatever the true explanation, in the initial periods of cultivation these factors simulate the mitogenetic effect.

The question of the specificity of the effect described above naturally arises.

To investigate the effect of certain serum proteins on the behavior of donor's lymphocytes a further seven experiments were carried out on the lymphocytes of five healthy donors (Fig. 2).

Addition of total γ-globulin, immune γ-globulin, antibodies against single-stranded DNA, and albumin isolated from the serum of a patient with systemic lupus erythematosus, in physiological concentrations into the blood serum of the healthy donors led in every case to a significant ($P < 0.001$) but different increase in the intensity of luminescence. Addition of PHA to the culture medium containing the appropriate component as mentioned above, however, caused either the active extinction of luminescence or no reaction to the mitogen.

It can accordingly be concluded from these results that the lymphocytes both of patients with systemic lupus erythematosus and of healthy patients to whose serum other components of heterologous origin had been added responded in a similar manner: by an increase in spontaneous activation and by absence of response to the action of the mitogen. These results are probably even more remarkable because the magnitude and character of activation of the lymphocytes from patients with systemic lupus erythematosus were independent of the titer of antibodies against single-stranded DNA inthe patients' serum (the titer in the pateints's serum varied from 1/60 to 1/640). No correlation likewise was found between the magnitude and character of the luminescence and treatment of the patients with corticosteroids and immunodepressants. Finally, there was no correlation between the content of γ-globulin fractions in the patients' serum, the intensity of luminescence of the lymphocytes, and the character of their response to PHA.

It can be concluded from these results and analysis of data in the literature [8] that lymphocyte chromatin is an extremely labile system which responds by a sharp modification to the action of a wide range of agents which are exogenous relative to the lymphocyte.

The universal nature of the response of lymphocytes to these factors offers hope of success in determining the nature or, at least, the localization of agents responsible for changes in the chromatin in certain hereditary diseases.

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