EFFECT OF BLOOD SERUM FROM SCHIZOPHRENICS AND HEALTHY DONORS ON DNA SYNTHESIS IN LYMPHOCYTES

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The number of DNA-synthesizing lymphocytes was determined in cultures of peripheral blood of patients with schizophrenia stimulated by phytohemagglutinin (PHA). It was shown that the number of lymphocytes capable of synthesizing DNA after the addition of PHA was 18% less in cell cultures from schizophrenics than in those from healthy donors. Incubation of healthy donors' lymphocytes in the serum of schizophrenic patients reduced the number of lymphocytes capable of synthesizing DNA in the presence of PHA by 21%.

KEY WORDS: schizophrenia; lymphocytes; DNA synthesis; phytohemaglutinin.

Peripheral blood lymphocytes from patients with schizophrenia have been shown to possess certain physiological properties characteristic of activated lymphocytes [2, 4]. Meanwhile it has been shown that the reaction of lymphocytes of schizophrenics to phytohemagglutinin (PHA) is weakened [1, 3]. The reduced response of the blood cells of schizophrenic patients to the stimulating action of PHA is due either to a weakened response of all cells to PHA or to the presence of a subpopulation of cells incapable of activation by PHA among the lymphocytes of patients with schizophrenia.

This paper describes an analysis of the whole lymphocyte population of patients with schizophrenia for ability to respond by DNA synthesis to stimulation by PHA. Another important task was to discover the causes of the reduced response of the cells to PHA. The existence of biologically active factors found in the serum of schizophrenic patients [5, 7] suggested that the weakened response of the lymphocytes may be due to the action of these factors. The effect of the blood serum of schizophrenic patients on the ability of healthy donors' lymphocytes to synthesize DNA was therefore studied also.

EXPERIMENTAL METHOD

White blood cells were obtained as described in [6]. The initial suspension was diluted with Eagle's medium to a concentration of $1 \cdot 10^6$ cells/ml. The concentration of autologous serum in the incubation medium in all experiments was 20%. The medium with cells was poured in volumes of 1 ml into sterile penicillin flasks. Before incubation of the cells PHA (PHA-P, Difco, USA) was added in the concentration of 40 μg/ml of the final medium.

Colchicine (Merck, West Germany) was used in a working concentration of 0.05 μg/ml. After incubation at 37°C for 1, 72, 96, and 120 h the contents of the flasks were collected, fixed, and stained by Feulgen's method; by means of a scanning cytophotometer (Opton, West Germany) the percentage of cells with an increased DNA content was determined. The methods of fixation, staining, measurement of the specimens, and statistical analysis of the results were described in [9].

In experiments to study the action of schizophrenic patients' serum on healthy donors' lymphocytes, white blood cells were separated from the autologous plasma, resuspended in an equal volume of the schizo-

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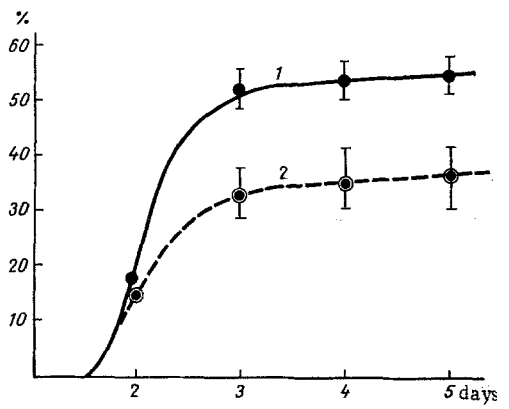

Fig. 1

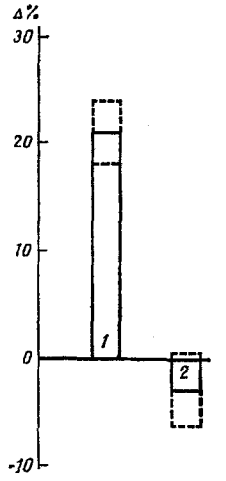

Fig. 2

Fig. 1. Number of DNA-synthesizing lymphocytes in PHA-stimulated cultures of blood from schizophrenics and healthy donors. Abscissa, time of cultivation of cells (in days); ordinate, % of DNA-synthesizing lymphocytes; 1) lymphocytes of healthy donors; 2) lymphocytes of patients with schizophrenia.

Fig. 2. Effect of serum of schizophrenic patients and healthy blood donors on number of DNA-synthesizing lymphocytes in PHA-stimulated blood cultures. Ordinate, difference between number of DNA-synthesizing lymphocytes in PHA-stimulated blood cultures containing autologous serum and number of these cells in cultures containing serum of blood donors. 1) Action of serum of schizophrenics on lymphocytes of healthy donors (n = 12); 2) action of healthy donors' serum on lymphocytes of schizophrenics (n = 11).

TABLE 1. Content (in %) of DNA-Synthesizing Lymphocytes in PHA-Stimulated Cultures of Blood Cells from Schizophrenic Patients and Healthy Donors (M ± m)

Time of incubation, days	Group tested		P
	schizophrenic patients	healthy donors	
3	33,2±4,5	52,0±3,6	<0,01
4	35,9±5,9	53,8±3,5	<0,02
5	35,9±5,7	54,6±3,4	<0,01

phrenic patient's serum for testing, and diluted to a final concentration of $1 \cdot 10^6$ cells/ml and 20% serum, respectively. Lymphocytes from schizophrenic patients were similarly transferred into healthy donors' serum. In this series of experiments no colchicine was used and the cultures were incubated for 72 h. Cultures of healthy donors' lymphocytes and of schizophrenic patients in a medium containing 20% of autologous serum were used as the control. The percentage of cells with an increased DNA content in the presence of PHA was determined by the method described above.

Altogether 28 patients with various forms of schizophrenia and 29 healthy donors were investigated.

EXPERIMENTAL RESULTS

To account for all lymphocytes of the population which responded during 5 days in culture to PHA stimulation by DNA synthesis, it was necessary to block division of the lymphocytes throughout the period of culture. For this purpose, in preliminary experiments the action of colchicine on PHA-stimulated lymphocytes was studied. In a concentration of 0.05 μg/ml colchicine completely prevented division of the lymphocytes, and cells with an increased DNA content remained in the culture for 5 days. Comparison of the values reflecting the action of colchicine showed that this substance equally prevented division of lymphocytes from schizophrenic patients and healthy blood donors.

The study of DNA synthesis showed that most peripheral blood lymphocytes ceased to synthesize DNA by the third day of incubation in response to stimulation by PHA. The results given in Fig. 1 in fact show that in cultures of white blood cells from schizophrenics and healthy donors the number of lymphocytes with an increased DNA content reached a maximum on the third day and therefore did not change significantly. The number of DNA-synthesizing lymphocytes (Table 1) in cultures of cells from schizophrenics was smaller than in cultures of healthy donors' cells, the difference being highly significant.

It will be clear from Fig. 2 that the blood serum of schizophrenics reduced by 21 ± 3% the number of DNA-synthesizing lymphocytes in cultures of white blood cells of healthy donors, whereas the blood serum of the healthy donors caused no significant change in the number of DNA-synthesizing lymphocytes in blood cultures of schizophrenic patients.

It can thus be concluded from the results that the reduced response of lymphocytes of schizophrenics to PHA is due to the presence of a subpopulation of cells incapable of activation by PHA. The number of cells responding to PHA by DNA synthesis was 18% less in cultures from schizophrenics than in those from healthy donors. These results are in harmony with the reduction in the number of lymphocytes of healthy donors responding to PHA in the presence of serum from schizophrenics. In fact, about 20% of lymphocytes of healthy donors, mixed with serum from schizophrenic patients, lose their ability to synthesize DNA in the presence of PHA. This can be explained by injury to a certain proportion of the peripheral blood lymphocytes of schizophrenics as a result of the action of serum factors on them. These injurious factors in the serum may include a membranotropic factor, the existence of which in patients with schizophrenia has been demonstrated by a number of workers [5, 7], as well as antithymocytic antibodies, also found in the serum of schizophrenics [8].

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