OSMOTIC RESISTANCE AND ADHESIVENESS OF PERIPHERAL BLOOD LYMPHOCYTES OF NORMAL HUMANS AND PATIENTS WITH SCHIZOPHRENIA

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Investigation of the osmotic resistance of peripheral blood lymphocytes from healthy blood donors and patients with schizophrenia revealed the existence under normal conditions of lymphocytes which differ sharply from one another in the criterion of resistance to osmotic shock: 20% of lymphocytes have low resistance and 80% high resistance. Lymphocytes with low resistance from healthy donors were shown to have increased adhesive properties. In 60% of schizophrenics there were virtually no lymphocytes with low resistance. The highly resistant lymphocytes of such patients include some characterized by abnormal resistance.

KEY WORDS: lymphocytes; resistance; schizophrenia.

In certain pathological states changes are observed in the resistance and adhesive properties of peripheral blood lymphocytes (PBL) [6-8, 10]. These parameters reflect the properties of the plasma membrane of lymphocytes and, for that reason, they must be interconnected in a certain manner. The object of this investigation was to assess the resistance of PBL by the use of a modification of a method widely used in work with erythrocytes. To establish interconnection between osmotic resistance and adhesiveness of lymphocytes in this investigation a method of removal of adhesive cells followed by analysis of the resistance of the residual nonadhesive fraction of lymphocytes was used.

PBL from healthy donors and patients with schizophrenia served as the test object. The PBL of schizophrenics are known [2-5] to have a plasma membrane with certain distinguishing features.

EXPERIMENTAL METHOD

The PBL of 13 healthy donors and 21 patients with schizophrenia were studied. To obtain a pure population of lymphocytes, human white blood cells were purified in a Ficoll–Isopaque system (purity over 90%). To obtain the nonadhesive fraction of lymphocytes, plasma with white blood cells was passed through a column with glass beads [9]. The yield of lymphocytes from the column when lymphocytes from healthy donors were tested was 68 ± 3%, when lymphocytes from schizophrenics was tested 57 ± 4%. The purity of the lymphocyte fractions obtained was about 95%.

The resistance of the cells was determined by exposing the lymphocytes to solutions of different ionic strength, followed by determining the number of undestroyed cells and the relative proportions of living and dead cells among them. For this purpose PBL, purified by the methods described above, were added in a volume of 0.1 ml in 1.0 ml of a solution prepared by diluting Hanks' solution with distilled water in the ratios of 10:0, 9:1, and so on, down to 0:10. After 1-2 min, the number of residual cells was counted in a Goryaev's chamber under the phase-contrast microscope. Dead cells were differentiated by staining with trypan blue, which was added to all solutions in 0.02% concentration.

EXPERIMENTAL RESULTS

A study of the resistance of PBL of healthy donors revealed the existence of lymphocytes which differed sharply in their resistance to hypoosmotic shock. It will be clear from Fig. 1 that 18 ± 2% of lymphocytes

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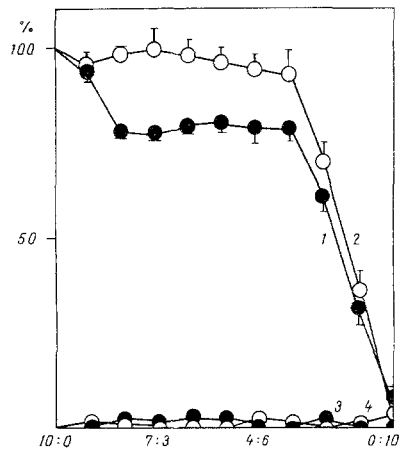

Fig. 1

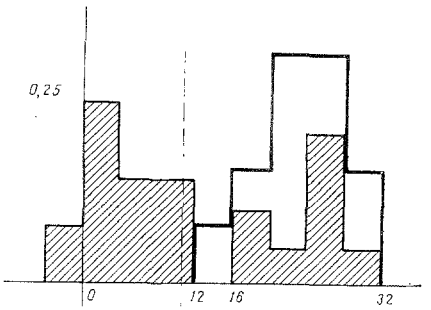

Fig. 2

Fig. 1. Effect of hypoosmotic treatment on blood lymphocytes from healthy donors. Abscissa, dilutions of Hanks' solution; ordinate, number of cells (in %). 1) Percentage of living lymphocytes in population purified in Ficoll–Isopaque solution (adhesive and nonadhesive lymphocytes); 2) percentage of dead cells in population of lymphocytes purified in Ficoll; 3) percentage of living cells in population of lymphocytes purified on column with glass beads (nonadhesive cell fraction); 4) percentage of dead cells in population of lymphocytes purified on column with glass beads.

Fig. 2. Distribution of donors and schizophrenics by relative percentage of lymphocytes with low resistance. Abscissa, number of subpopulation of lymphocytes with low resistance (in %); ordinate, frequency (n). Shaded area represents patients with schizophrenia, unshaded area healthy donors.

are destroyed after minimal dilutions of Hanks' solution (from 10:0 to 8:2). The remaining cells did not begin to be destroyed until exposed to much stronger hypoosmotic treatment (dilutions of 2:8 to 0:10). Under these circumstances, the number of dead cells among the lymphocytes which remained undestroyed at this stage of dilution did not exceed 2-4% (Fig. 1).

Analysis of individual curves of resistance of healthy donors' blood lymphocytes and of the histogram of distribution showed that the distribution of the donors' lymphocytes was characterized by great constancy, and the histogram of distribution of the donors was monomodal, close in shape to the curve of the normal distribution (Fig. 2).

During passage of plasma with leukocytes of healthy donors through a column with glass beads, lymphocytes with increased adhesive properties were held up on the column (32%). A study of the resistance of nonadhesive lymphocytes revealed absence of lymphocytes with low resistance in the nonadhesive fraction (Fig. 1). Consequently, lymphocytes from healthy donors with low resistance are characterized by increased adhesiveness.

A different picture was found during the study of the schizophrenics. When the whole lymphocyte population of the patients was studied a conspicuous feature was the high variability of the number of lymphocytes with low resistance (Fig. 2). It will be clear that the patients studied formed two clearly demarcated groups. One group (40% of those tested) was indistinguishable from normal in its percentage of cells with low resistance, and the resistance curve for these patients coincided with that characteristic of healthy donors. In the other group of patients (60% of those tested) the proportion of cells with low resistance was much reduced, being only 2 ± 2%, i.e., about one-tenth of that normally found. As an alternative index for separating the patients into two groups, the parameter $M_h - 2\sigma$ was used (where M_h is the arithmetic mean relative percentage of lymphocytes of low resistance in the blood of healthy subjects, and σ is the standard deviation). The resistance curve of blood lymphocytes of a patient with schizophrenia, typical of the patients of the second group, is illustrated in Fig. 3. Clearly the lymphocytes of such patients start to break up only in a dilution of 3:7, i.e., in such patients lymphocytes with low resistance are virtually absent.

In this experimental situation one other distinguishing feature of the patients' PBL came to light: During exposure to Hanks' solutions in dilutions of between 4:6 and 0:10, among the undestroyed cells there was a

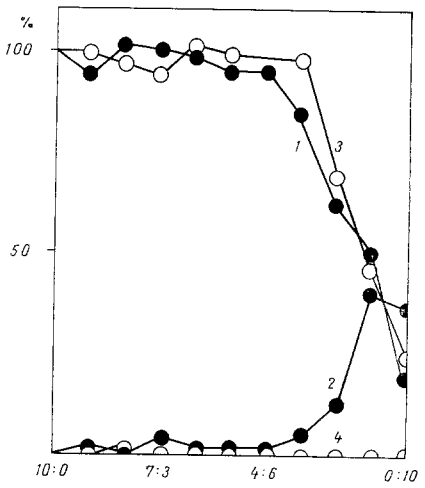

Fig. 3. Blood lymphocytes from a patient with schizophrenia exposed to hypoosmotic treatment of different strengths. Legend as in Fig. 1.

high proportion of dead cells. The greatest number of dead cells was found in a dilution of 1:9, when it was 24±7% on average for all patients. The difference from the donors with respect to this parameter was highly significant ($t_d = 3.29$, $P < 0.01$). The character of correlation between these two features distinguishing the behavior of the patients' cells during exposure to hypoosmotic treatment has not yet been explained. Direct correlation between the number of lymphocytes with low resistance and a parameter reflecting the number of dead cells could not be found ($r = -0.313$, $P < 0.1$).

Passage of blood cells from schizophrenic patients through a column followed by determination of their resistance showed that the column completely retained lymphocytes which, in the corresponding dilutions (3:7 to 0:10) formed so-called dead cells. It was thus concluded from a comparison of data on resistance and adhesiveness of PBL of schizophrenic patients and healthy donors that the adhesive lymphocytes of the patients and donors belonged to different cell subpopulations. This conclusion is in good agreement with previous observations relating to stimulation of PBL of schizophrenic patients with mitogens [1].

All the facts examined above, together with data showing that the total number of lymphocytes in the peripheral blood of patients with schizophrenia is the same as normally found [4], are evidence of a sharp decrease in the number of lymphocytes with low resistance and of the formation of cells which die without disintegration during exposure to relatively strong osmotic treatment.

The method used in this investigation to study the resistance of human PBL proved to be effective for detection and quantitative evaluation of some features distinguishing the lymphocytes in schizophrenia. Accordingly the writers consider that this method can be applied to the study of certain other pathological states.

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