

BLAST-TRANSFORMATION OF LYMPHOCYTES IN CHRONIC LIVER DISEASES

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The immunological activity of the circulating lymphocytes was studied in 36 patients with chronic liver diseases by means of the blast-transformation reaction in vitro in the presence of phytohemagglutinin (PHA) and other stimulators. The effect of prednisolone and 6-mercaptopurine on transformation of the lymphocytes was determined. In patients with chronic hepatitis and cirrhosis of the liver inhibition of blast-transformation of the lymphocytes was observed in the presence of PHA, evidently indicating a decrease in their immunoreactivity. In some patients with these diseases the lymphocytes responded to DNA and liver homogenate. In patients receiving immunodepressants, blast-transformation of the cells in culture was reduced to a certain degree. The possibility of evaluating the effectiveness of immunodepressant therapy by means of the blast-transformation reaction is discussed.

Autoimmune mechanisms play an important role in the pathogenesis of chronic diseases of the liver and, in particular, of certain forms of cirrhosis [4].

The object of the present investigation was to study blast-transformation of circulating lymphocytes in vitro in the presence of phytohemagglutinin (PHA) and other stimulators in chronic diseases of the liver.

EXPERIMENTAL METHOD

Cultures of circulating lymphocytes from 36 patients aged from 18 to 56 years were investigated. Of this number 16 patients had postnecrotic active cirrhosis of the liver during a flare-up and 3 were in a phase of remission, 12 patients had biliary cirrhosis of the liver during a flare-up, including 9 with primary and 3 with secondary forms, and 5 patients had a flare-up of chronic hepatitis. The diagnosis in all patients was confirmed by laparoscopy, liver scanning, and other instrumental methods of investigation. As a rule the indices of the liver function tests were altered. In 6 patients the progressive course of the disease led to death; 17 patients received corticosteroids and 3 patients received 6-mercaptopurine. Twelve patients of this group were investigated before and after treatment and 8 patients were investigated during prolonged (2-3 years) treatment with prednisolone.

Besides the patients with chronic liver diseases, patients with other diseases not affecting the liver, and also clinically healthy donors (35 persons) were investigated.

Cultures of lymphocytes were prepared by Hirschhorn's method [3]. The cells were grown on cover slips in penicillin flasks in medium No. 199 with 15-20% autologous plasma or with fresh human group 0 (I) donor's plasma, inactivated at 56°C. The number of transformed cells was counted in 500 cells in fixed and stained films.

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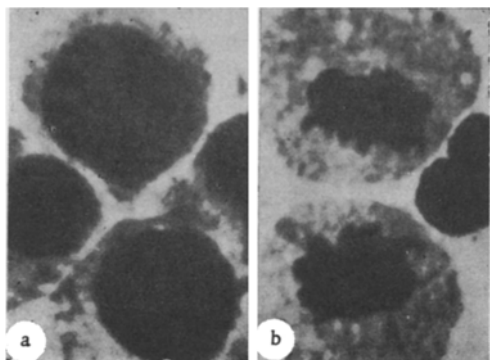


Fig. 1. Transformation of lymphocytes in vitro: a) lymphoblasts and lymphocytes; b) mitosis. Azure-eosin, 420 \times .

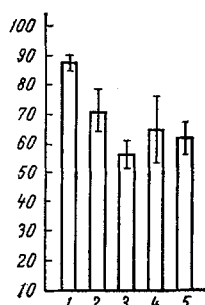


Fig. 2. Blast-transformation of lymphocytes of patients with chronic liver diseases. Ordinate, mean indices of blast-transformation (in %) with standard deviation; abscissa: 1) normal; 2) postnecrotic active cirrhosis in the flare-up period; 3) postnecrotic active cirrhosis in the remission period; 4) active biliary cirrhosis of the liver; 5) chronic hepatitis.

Phytohemagglutinin (PHA) (M, Difco) in a dose of 0.01 ml/ml leukocyte suspension was used as nonspecific stimulator in 36 patients; all tests were carried out with the same batch of PHA. Native DNA from calf thymus in a concentration of 1-2 μ g/ml was used as the nonspecific stimulator in 20 patients, and liver extract made up in physiological saline (1:10), with a protein concentration of between 50 and 100 mg%, was used in 10 patients. The extract was prepared from the liver tissue of patients with chronic diseases of the liver undergoing operation. Besides cultures to which the stimulators were added, other controls without stimulators were set up.

EXPERIMENTAL RESULTS

The largest number of transformed cells was observed on the 3rd-4th day of cultivation of the lymphocytes with PHA, DNA, or liver extract. Mitosis was frequently observed in the transformed cultures (Fig. 1). Spontaneous transformation of lymphocytes into macrophages of fibroblasts was observed in the cultures without stimulators. Cultures of cells obtained from patients with chronic diseases of the liver were often unstable.

It will be clear from Fig. 2 that the functional activity of the lymphocytes was slightly reduced in the patients with chronic hepatitis and cirrhosis of the liver. The indices of blast-transformation of the lymphocytes varied in these patients from 30 to 87%. No definite relationship could be found between activity of the lymphocytes in this reaction and the clinical or pathological form of the liver disease. The highest intensity of the reaction (72-87%) was observed in only 3 patients seriously ill with a rapidly progressive "juvenile" cirrhosis and with primary biliary cirrhosis of the liver, who died soon after from hepatic failure.

In parallel tests in which the autologous plasma was replaced by homologous the results of the blast-transformation reaction of the lymphocytes were unchanged (18 patients).

In response to DNA a blast-transformation reaction of the lymphocytes was observed in nearly half of the patients tested (in 9 of 20), mainly in patients with postnecrotic cirrhosis of the liver or with chronic hepatitis in the active phase (on the average up to 18% of blast cells). When liver extract was used a positive reaction was obtained in only 3 patients with cirrhosis of the liver during a flare-up (18, 14, 8%, respectively). Lymphocytes of the donors of the control group (25 subjects) did not react to these substances.

Investigation of the dynamics of the lymphocyte reaction after treatment with PHA during immunodepressant therapy revealed significant differences: in 6 patients marked inhibition of the reaction was found, in 4 there was no change, and in 2 there was an increase in blast-transformation (Fig. 3).

Comparison of the effectiveness of immunodepressant therapy with the activity of the lymphocytes in the blast-transformation reaction in vitro in 20 patients showed that clinical improvement was accompanied by a decrease in the immunoreactivity of the lymphocytes in vitro.

Prednisolone therapy led to a decrease in the reaction of the lymphocytes to DNA.

These investigations showed some decrease in the functional activity of the lymphocytes in the blast-transformation reaction in patients with chronic hepatitis and cirrhosis of the liver compared with the

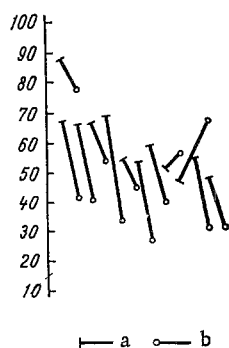


Fig. 3. Effect of immunodepressants on blast-transformation of lymphocytes in vitro. Ordinate, number of cells transformed into blast cells (in %), before treatment (a) and after treatment (b).

uncertain. The available data on this question have usually been obtained by the study of small heterogeneous groups of patients, with the result that contradictory conclusions have been drawn [1, 6].

It can be postulated on the basis of data in the literature and the results described above that the phenomenon of transformation of lymphocytes in vitro under the influence of different stimulators may reflect to some extent an immunological reaction carried out by immunocompetent cells. The fact that a positive reaction to DNA is found most frequently in patients with an active pathological process in the liver can perhaps be explained on the grounds that destruction of the tissue is accompanied by the release of free DNA into the blood stream, and when this DNA is incorporated into immunologically active complexes it sensitizes the patient's lymphocytes or plays the role of an adjuvant.

The extent of the decrease in immunological reactivity of the lymphocytes in patients receiving immunodepressants varied from individual to individual. Better results of immunodepressant therapy were found in patients in whom blast-transformation of the lymphocytes was sharply reduced in the course of treatment (Fig. 3). Consequently, the immunoreactivity of the lymphocytes can be modified by chemotherapy. The response of lymphocytes in vitro to antigen in the course of immunodepressant therapy can be used as an indicator of the effectiveness of treatment.

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