CHANGES IN Ca,Mg-DEPENDENT DNA ENDONUCLEASE IN ISOLATED HUMAN LYMPHOCYTE NUCLEI IN LYMPHOPROLIFERATIVE DISEASES

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UDC 616.155.392-07:616.155.32-008.92

Involvement of immunocompetent cells in the tumor process is inevitably accompanied by a disturbance of their functional activity, manifested at the whole-body level as a state of secondary immunodeficiency. To detect the immunodeficiency in lymphoproliferative diseases (LPD) surface markers of populations and subpopulations of T- and B-lymphocytes are used. One parameter of malignant transformation of lymphoid cells is determination of differential or tumor-associated surface antigens [10]. The clinical value of these methods is no longer in dispute.

Meanwhile changes observed in the phenotype and functional properties of lymphocytes are a reflection of the profound reorganization of their metabolism and, in particular, of their genome functioning systems. Investigation of changes taking place in these systems and, in particular, changes in enzymes of nucleic acid metabolism, responsible for functioning of the genome, may be one approach to the study of the mechanisms of malignant cell transformation. It has recently been shown that lymphoproliferative processes are accompanied by changes in the activity of many DNA-enzymes in lymphoid cells and, in particular, enzymes of purine metabolism [7], DNA-ligase [9], and DNA-polymerases α and β [8], as well as changes in activity of serum alkaline DNase [6]. Determination of activity of certain DNA-enzymes has been suggested as enzymologic marker of transformed lymphocytes.

The writers showed previously that chronic lymphatic leukemia (CLL) can be accompanied by a decrease in the intensity of Ca,Mg-dependent endonucleolysis of the DNA of peripheral blood lymphocytes [2]. This paper describes a study of the intensity of Ca,Mg-dependent DIFA endonucleolysis in different clinical forms of human LPD, and also in normal individuals and in patients with various diseases of nontumor nature.

The results show that the intensity of Ca,Mg-endonucleolysis may be a valuable indicator of malignant transformation of lymphocytes.

EXPERIMENTAL METHOD

Altogether 89 persons were studied, including 26 children aged from 3 months to 14 years. Control group consisted of 25 normal individuals: 22 blood donors and 3 children. Of 55 patients with LPD 13 had a clinical diagnosis of CLL, 19 had malignant non-Hodgkin's lymphoma (MNHL), and 23 had lymphogranulomatosis (LGM).

Peripheral blood mononuclears were obtained by the method in [5], and their cell nuclei were isolated by the method in [4].

The isolated cell nuclei were incubated in buffer containing 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM $CaCl_2$, and 0.25M sucrose, pH 8.1-8.3, at 37°C for 1.5 h. Each sample contained 5 mg (as DNA) of cell nuclei. The intensity of Ca,Mg-dependent endonucleolysis was determined by the method in [2] with the following modifications: electrophoresis of DNA was carried out

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 6, pp. 543-546, June, 1990. Original article submitted July 31, 1989.

0007-4888/90/0006-0720$12.50

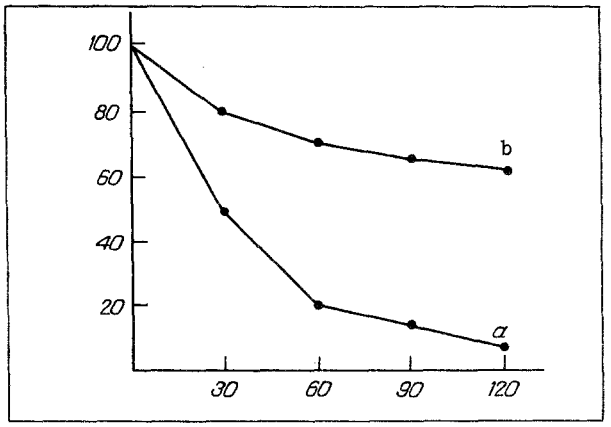

Fig. 1. Intensity of degradation of chromosomal DNA in normal individual (a) and in CLL (b). Abscissa, incubation time of nuclei (in min); ordinate, amplitude of peak of substrate DNA (in percent).

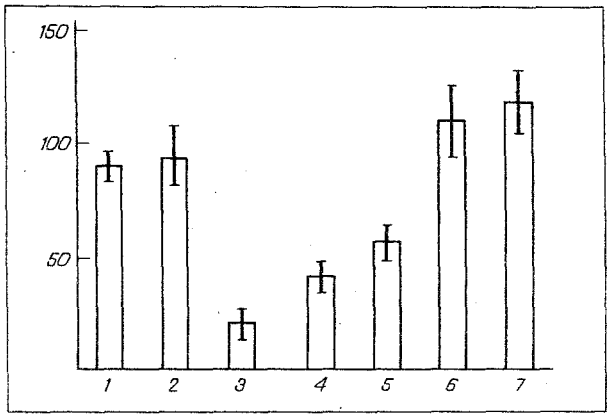

Fig. 2. Intensity of Ca,Mg-dependent endonucleolysis of lymphocyte DNA in groups of patients tested. Here and in Fig. 3, ordinate: intensity of endonucleolysis (in units). 1) Normal blood donors, 2) Healthy children, 3) CLL, 4) MNHL, 5) LGM, 6) Pathology of nontumor nature, 7) Remission of MNHL.

in 0.7% agarose gel, and the change in the ratio of the peak width of substrate endogenous DNA at the height of 2/3 of its amplitude to that at 1/3 of its amplitude was used as measure of the intensity of endonucleolysis. The unit of intensity was taken to be the change in this ratio after 1.5 h of incubation of the sample under standard conditions by 0.01 compared with the control.

To characterize the immune status of patients with LPD the number of T- and B-lymphocytes (E-, EA-, and EM-rosette formation) and the ratio of helper T cells/suppressor T cells in the system (assessed as the level of T cells carrying Fc-receptors to IgM and IgG) were determined [1].

EXPERIMENTAL RESULTS

During incubation of the isolated lymphocyte nuclei in the presence of Ca^{2+} and Mg^{2+}, Ca,Mg-dependent endonuclease was activated, leading to degradation of endogenous DNA. Comparison of the kinetics of this process in healthy blood donors and in patients with CLL shows that in the latter case Ca,Mg-dependent endonucleolysis was significantly delayed (Fig. 1).

These observations served as the basis for comparison of the intensity of Ca,Mg-dependent endonucleolysis of DNA in persons with different forms of LPD. The results of this study are given in Fig. 2.

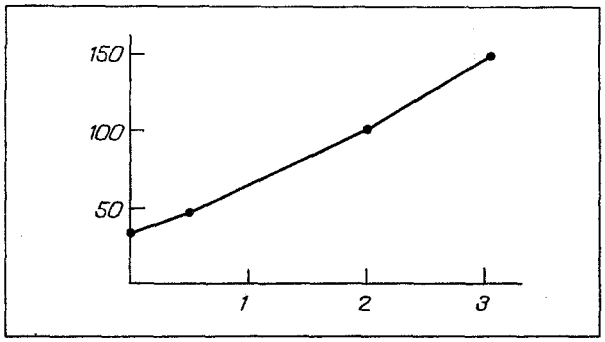

Fig. 3. Time course of changes in intensity of Ca,Mg-dependent DNA endonucleolysis during treatment of patient with LGM by standard chemotherapy courses. Abscissa, duration of treatment (in months).

The intensity of Ca,Mg-dependent endonucleolysis of DNA of normal lymphocytes was 91 ± 7 units, with a range of variation of between 50 and 136 units for the blood donors and 94 ± 14 units in children. All forms of LPD studied were accompanied by a decrease in the intensity of Ca,Mg-dependent endonucleolysis. This decrease was most marked in the case of CLL: 20 ± 7 units, with a range of variation from 0 to 56 units. The intensity of endonucleolysis observed in the case of MNHL and LGM was a little higher: for MNHL it was 38 ± 8 units and varied from 2 to 73 units, for LGM it was 58 ± 9 units (from 1 to 102 units). Statistical analysis by Student's test revealed that the changes discovered in the intensity of endonucleolysis in CLL and MNHL were significant compared with the normal level ($p < 0.001$).

As a further control group, we studied persons with nontumor pathology. This group consisted of three patients with thyroid gland pathology (Hashimoto's goiter), 2 patients with diabetes mellitus, 2 with gynecologic and 2 with infectious diseases (acute dysentery and acute salmonellosis). The intensity of Ca,Mg-dependent endonucleolysis of the lymphocyte DNA from these patients was 112 ± 18 units, with a range of variation from 55 to 200 units, i.e., it was virtually indistinguishable from normal values.

Cases of reduced intensity of Ca,Mg-dependent endonucleolysis (under 40 units) were observed only in LPD. The prevalence of these cases was highest in CLL, for which it was 88%. In MNHL and LGM a decrease in the intensity of endonucleolysis was discovered in 82 and 41% of observations respectively. Thus the decrease in the intensity of Ca,Mg-dependent endonucleolysis of lymphocyte DNA in CLL and MNHL can be considered to be a sufficiently regular phenomenon.

Parallel with the study of the intensity of endonucleolysis in patients with LPD we also determined some parameters of their immune status: the relative content of E^+-cells (T) and of EAC^+-cells (B) and the ratio of helper/suppressor T cells. In CLL and MNHL the B-type of cell proliferation was discovered, in LGM the T-type (in 75% of cases) and the O-type (in 25% of cases). On the whole, disturbances in LPD were accompanied by an imbalance of the immunoregulatory cells in favor of strengthening of the suppressor potential.

The intensity of Ca,Mg-dependent endonucleolysis, like the immunologic parameters, changed toward normal during successful treatment of patients with LPD, and with the onset of a clinical remission. Patient G-li (with LGM), studied before treatment and during 3 months of treatment, is demonstrative in this respect. The trend of the change in the intensity of endonucleolysis of DNA in the lymphocytes of this patient is demonstrated in Fig. 3, which shows that during the treatment of this patient the intensity of endonucleolysis rose to the normal level.

Altogether we determined the intensity of Ca,Mg-dependent endonucleolysis in 14 patients with LPD in the remission stage (5 patients with CLL, 3 with MNHL, 6 with LGM). These patients were treated by standard courses of chemotherapy, using tactivin for the treatment of LGM and reaferone for the treatment of CLL (fibrocellular leukemia). The state of remission in 93% of the patients studied was accompanied by restoration of the intensity of endonucleolysis to normal values (Fig. 2): the mean intensity of endonucleolysis was 120 ± 15 units.

The results thus demonstrate the regular character of a decrease in the intensity of Ca,Mg-dependent endonucleolysis of lymphocyte DNA in the clinical forms of LPD examined above and, in particular, in the case of CLL. These findings agree with the results of a study of human lymphocytes obtained by the writers previously [2]. Moreover, similar results also were obtained in a study of bovine lymphocytes from animals with spontaneous and experimental CLL [3]. On the basis of these results the decrease in the intensity of Ca,Mg-dependent endonucleolysis can be regarded as a marker of malignant transformation of lym-

phocytes. Other workers who studied changes in DNA-endonucleases in malignant cell transformation came to similar conclusions [6]. Meanwhile neither the origin, the type, or the intracellular location of these enzymes has been determined in any of the investigations of human DNA-endonucleases during malignant transformation with which we are familiar.

Changes which we observed in the intensity of DNA endonucleolysis are most probably connected with changes in activity of nuclear Ca,Mg- dependent endonuclease. This was shown by our attempts to compare activity of this enzyme in protein extracts of human lymphocyte nuclei from healthy blood donors and patients with CLL, and also the similar results obtained in experiments on cattle [3].

The results for reactivation of Ca,Mg-dependent DNA endonucleolysis of lymphocytes from patients with LPD, with the onset of a clinical remission, are in good agreement with existing data [6], although in the latter case the authors studied serum DNase of unconfirmed origin. This suggests that determining the intensity of Ca,Mg-dependent endonucleolysis of lymphocyte DNA is of prognostic value during the treatment of patients with different forms of lymphoproliferative diseases.

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