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COMPARATIVE STUDY OF DNA BREAKS IN HUMAN LEUKEMIC AND NORMAL BLOOD LEUKOCYTES

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The action of ionizing radiation, carcinogens, and oncogenic viruses is known to be accompanied by fragmentation of the DNA of the normal cell. Diseases due to genetic defects of the DNA repair system, such as xeroderma pigmentosum, are characterized by an increase in the frequency of tumor development. Lymphocytes of patients with primary and secondary aplastic anemia are known to have more single-stranded DNA breaks than healthy human lymphocytes [5].

With these facts in mind, the level of single-strand DNA breaks was compared in peripheral blood leukocytes from healthy subjects and from patients with chronic lymphatic and myeloid leukemia.

EXPERIMENTAL METHOD

Lymphocytes and granulocytes from the peripheral blood of healthy donors served as the control. Lymphocytes were isolated by Böyum's method [3] using a mixture of Ficoll and Urotrast with a specific gravity of 1.08. To isolate granulocytes, the Ficoll–Urotrast mixture was layered above an equal volume of polyvinol (mol. wt. 20,000) with a specific gravity of 1.12. The isolated lymphocytes or granulocytes were washed three times with cold physiological saline and the cells were separated by centrifugation for 10 min at 400 g. Single-strand DNA breaks were determined by the method of Fedorov and Borisov [1, 2]. A cell suspension (2×10^6) was lysed by addition of 4 volumes of a solution of the following composition: 2.5M NaCl, 0.025M EDTA-Na_3, 5 g/liter Na laurylsarcosinate; pH 9.0. The lysate was passed through a BS filter (from Millipore) by means of a peristaltic pump and washed with 5 ml 0.002M EDTA solution, pH 10.0. DNA was eluted with 0.1M NaOH at 40°C at the rate of 0.1 ml/min and four fractions each of 2.5 ml were collected;

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TABLE 1. Number of DNA Breaks in Blood Leukocytes of Patients with CLL and CML

Patient's initial	Diagnosis	Character of course of disease	Number of breaks in DNA of leukocytes
B			1,66
T.	CLL	Slow	2,17
N			1,90
R			3,73
K.			3,87
Ts	CLL	Rapid	4,25
S			2,85
Z			3,61
M.			12,60
L	CLL	Crisis	40,00
V.			6,87
D	CML	—	4,72
Kh			6,45

Legend. Number of breaks in DNA of healthy donors' leukocytes 1.25 ± 0.2. Number in granulocytes 3.85 ± 0.6.

one fraction was eluted at 55°C. DNA in the eluates was determined fluorometrically. For this purpose, the fractions of eluate were treated with 0.1 ml of a solution containing 130 g/l of ethidium bromide (EB) and 4M Na_2HPO_4, and the fluorescence of the eluates (F_k) was measured at an excitation wavelength λ = 365 nm ($\Delta\lambda_{exc}$ = 10 nm) and emission wavelengths λ = 600 nm ($\Delta\lambda_{em}$ = 20 nm); fluorescence of a control solution not containing DNA (F_0) also was measured. The DNA concentration in the eluates was calculated by equations:

$$[\text{DNA}] = 2{,}381\varphi + 0{,}342\varphi^2 + 0{,}077\varphi^3 \quad (1)$$

or

$$[\text{DNA}] = K_k \cdot \frac{\varphi}{Q - \varphi} + [\text{EB}]_\Sigma \cdot \frac{\varphi}{Q}, \quad (2)$$

where

$$\varphi = \frac{F_k}{F_o} - 1; \; Q = \frac{q_k}{q_o} - 1.$$

In these equations K_k is the constant of complex formation between DNA and EB; q_0 and q_k the quantum yield of the pure dye and the dye in the form of a complex with DNA respectively; Σ the total concentration of dye in the eluate. Values of the coefficients in equation (1) and the values of K_k and Q in equation (2) were obtained by processing the calibration data. The rate of elution of DNA has been shown [1, 2, 6] to be determined by the frequency of single-strand breaks. To determine more exact values of the rate of elution of DNA on the graphs, the ratio between the quantity of DNA eluted at 40°C and its high-molecular-weight fraction was plotted along the ordinate and the fraction number of time on an arbitrary scale along the abscissa. The elution profiles within these coordinates were independent of the number of lysed cells within comparatively wide limits.

The numerical data were subjected to statistical analysis on the "Alphatronic-332" minicomputer (from Diehl, West Germany), the arithmetic mean and standard dispersion for the results of n experiments be obtained as the results of the biological tests. The standard computer program was used for the operations. To determine coefficients and constants in equations (1) and (2) the results of calibration for 10 points were used. These constants were calculated by a program devised by the writers themselves, using the method of least squares from the condition of the minimum of the standard error of DNA concentration.

EXPERIMENTAL RESULTS

The results of individual investigations are summarized in Table 1 and the averaged results are given in Figs. 1 and 2. The smallest number of single-strand breaks, it will be seen, was found in DNA from the peripheral blood lymphocytes of healthy donors. DNA from leukocytes of patients with chronic lymphatic leukemia (CLL) with a slow rate of development contained about 1.5 times more single-strand breaks, and with CLL with a rapid course of development, 3 times more single-strand breaks (Fig. 1). DNA from peripheral blood leukocytes of patients with chronic myeloid leukemia (CML) had 4.4 times more single-strand breaks than DNA from normal lymphocytes (Fig. 2). However, recalling that the cells of granulocyte type

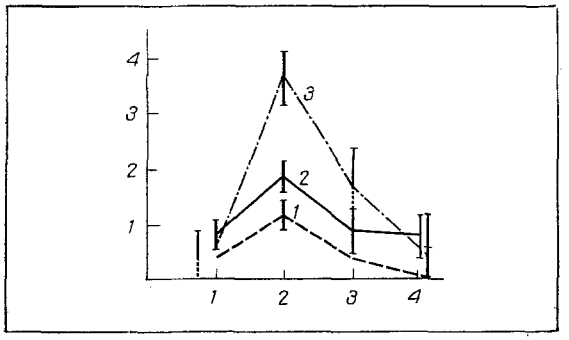

Fig. 1. Averaged curves of alkaline elution of DNA of lymphocytes, characterizing number of single-strand breaks: 1) healthy donors; 2) patients with CLL with slow rate of development of disease; 3) patients with CLL with rapid rate of development of disease. Here and in Fig. 2: abscissa, nos. of fractions; ordinate, ratio between DNA concentrations at 40°C and 55°C.

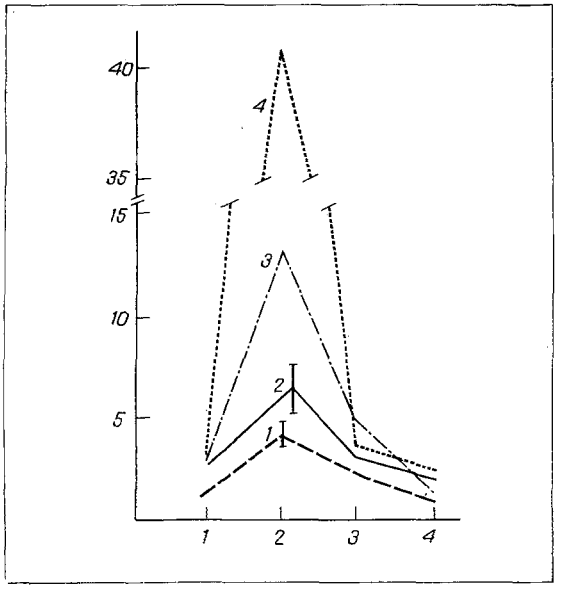

Fig. 2. Alkaline elution profiles of DNA from granulocytes and tumor cells, characterizing number of single-strand breaks: 1) granulocytes of healthy donors (statistical data); 2) granulocytes of patients with CML, 3 and 4) tumor cells during leukemic crisis terminating in death (individual cases).

predominate among leukocytes of patients with CML, it is evidently more correct to compare the results of determination of single-strand breaks in leukocytes of patients with CML with results relating to DNA of normal granulocytes. If this comparison is made, the number of single-strand breaks in DNA from leukocytes of patients with CML was 1.5 times greater than in DNA from granulocytes of healthy donors.

Granulocytes are known to be unable to divide, to have a significantly shorter life span than lymphocytes, and to have a nucleus which contains only heterochromatin and to be considerably repressed. For this reason the repair of injuries to DNA in granulocytes is evidently substantially weakened. This defect of DNA repair is also found in myeloid cells of patients with CML.

Two patients with CLL (L. and M.) had well-marked features of malignant changes (tumor cells and neoplasma, no effect from treatment). The number of DNA breaks in their lymphocytes was particularly high (more than 10 times greater than their number in DNA from normal lymphocytes), and this evidently reflected profound disturbances in the repair system of the lymphocytes.

In connection with the results showing the larger number of single-strand breaks in DNA in leukemic cells of patients with CLL and CML obtained during this investigation, it must be recalled that bone marrow cells of patients with aplastic anemia have regions of open (denatured) DNA at the metaphase, anaphase, and early telophase stages, whereas DNA of healthy donors' cells at these stages is completely supercoiled and contains no such regions. The number and extent of these regions determine not only the kinetics of DNA denaturation, but also the ability of chemical carcinogens, steroid hormones, and virus genomes to bind with it [4].

The increase in the number of single-strand breaks in DNA of leukemic cells may, on the one hand, be the result of a change in the system of repair enzymes and, on the other hand, an essential mechanism for maintaining the cell in the transformed state. In fact, according to existing data [7], the ability of leukocytes of patients with CLL to carry out repair is significantly reduced. It has recently been shown that induction of erythroid differentiation in cultures of erythroleukemic cells is accompanied by an increase in the number of DNA breaks [8, 9].

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