INTERPHASE CHROMATIN OF PERIPHERAL BLOOD CELLS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA (Ph^+)

I. É. Yudina and K. N. Federova

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The writers showed previously by cytofluorometry with acridine orange labeling [6] in the terminal cell DNP denaturation modification [3, 7] that curves showing dependence of AO binding with chromatin DNA of peripheral blood lymphocytes and granulocytes of normal individuals on temperature have the following regular features: In 40% of cases irrespective of sex, six peaks are obtained at definite temperatures (modal class), and in 60% of cases repetitive types of deviations (sex-dependent) are found, whose character permits several subgroups to be distinguished: five in the control group of women, seven in that of men (no fewer than five identical cases in each subgroup) [4].

These distinguishing features of the curves mentioned above (evidently indicating a change in the structural organization of chromatin) in patients with inborn chromosomal anomalies [3, 5] and correlations of these deviations with these characteristics of the curves in the separate subgroups of normal individuals (as possible genetic predisposition) make it essential to analyze the structural features of cell chromatin in other chromosomal aberrations also. In the investigation described below an analysis of this kind was undertaken on peripheral blood cells of patients with an acquired chromosomal anomaly, namely chronic myeloid leukemia, CML (Ph^+), during periods of blast crisis and clinical and hematologic compensation.

EXPERIMENTAL METHOD

Nuclear chromatin of peripheral blood cells of 25 patients with CML (Ph^+) aged between 16 and 60 years in different stages of the disease was studied. Changes in chromatin structure during heating (from 20 to 95°C) were recorded as the quantity of luminescent label — acridine orange (AO) bound after every 1-2.5°C. The investigations were conducted on cells incubated for 1 h in Eagle's nurtrient medium with the addition of 10% autologous serum.

The intensity of luminescence of AO bound with DNA of the cell chromatin was measured on an MSP-0.5 microscope-photometer (Opton, West Germany). Luminescence was excited by light with λ = 365 nm and recorded at λ = 530 nm.

The instrument, details of the experiments, and methods of isolation and culture of the peripheral blood cells were described previously [4].

Thermal denaturation of chromatin in the cell was carried out as described by Ringertz [7] in the writers' modification [4].

Melting curves of the cell chromatin were compared at all points of the curve in relation to average level of intensity of fluorescence and parallelness of the change in its intensity. The significance of differences was estimated by one version of two-factor dispersion analysis [1].

EXPERIMENTAL RESULTS

The curves chosen for analysis in the phase of blast crisis of the disease in untreated patients (number of blast cells in the peripheral blood 80-100%) had six maxima, of which the four first (unlike in the control) were located within temperature intervals that differed individually for each patient. The maximum at 85°C was absent, but one appeared at 82°C (Fig.

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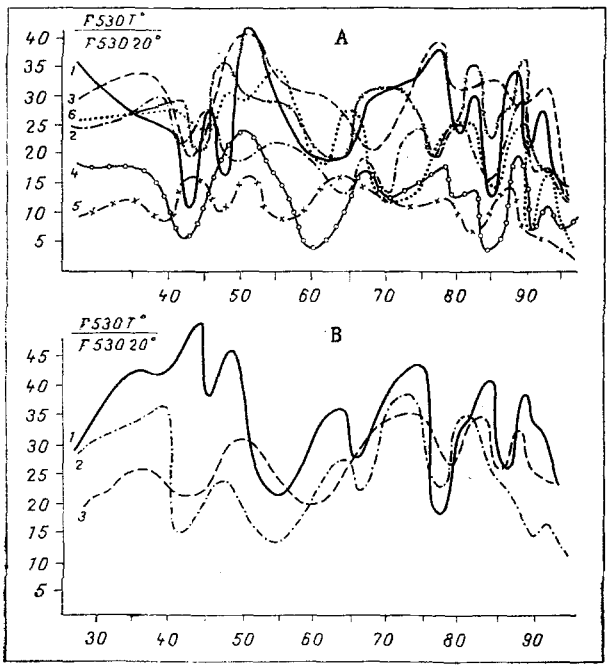

Fig. 1. Melting profiles of interphase chromatin of peripheral blood cells of patients with CML (Ph^+). A) Period of blast crisis before beginning of treatment (1, 2, 3, 4, 5 — individual cases), B) 1, 2, 3: the same patients during period of clinical and hematologic compensation. Abscissa, temperature (in °C); ordinate, ratio of intensity of fluorescence of AO bound of human peripheral blood cells (F = 530 nm) at T°C to that at 20°C: $F_{530}T°/F_{530}20°C$ ($0.2 \leq \sigma \leq 0.2$).

1). In our opinion these data are experimenal proof of a change in the supramolecular packing of the cell chromatin during the period of blast crisis of CML.

In the period of clinical and hematologic compensation (number of blast cells in the blood 5-20%) the melting curve of the cell chromatin of these same patients (17 of 25) had six maxima at definite temperatures: 45°C (±2), 65°C (±2), 78°C (±1), and 85, 88, and 92°C (±2), as is characteristic of healthy individuals in the modal class group (Fig. 1). The melting curves of the remaining individuals were not completely normal.

Two important factors, from our point of view, can be deduced from these results.

1. "Normalization" of the curves in cells of CML patients in the period of clinical and hematologic compensation of the disease. This, we may consider, indicates that the version of the luminescent fluoormetry method described above is a promising technique for use in the clinical management of hemoblastoses.

2. In the period of clinical and hematologic compensation three populations of peripheral blood cells were investigated: blast forms, small lymphocytes, and granulocytes. The corresponding curves for chromatin of both blast forms and mature cells (lymphocytes and granulocytes) were virtually identical. Since the writers showed previously [2] that the structural features of cell chromatin depend on the composition of the blood serum, it was suggested that the structural features of chromatin in cells of patients with CML depend on their microenvironment.

This hypothesis was tested in cell culture during crossed replacement of sera and peripheral blood cells of patients and normal individuals. Melting curves of chromatin of patients with CML in the blast crisis phase were obtained after preliminary incubation for 1 h in healthy human serum, and vice versa. Plasma and cells were obtained from five healthy donors aged from 18 to 50 years. During incubation of a patient's cells in healthy human serum, the ability of chromatin DNA to bind AO underwent a partial change in the same way as is characteristic of chromatin from healthy human cells (Fig. 2). In turn, the chromatin melting curves of healthy human cells, when incubated in the serum of a patient with CML, also were changed and were similar to those for cells of the corresponding patient (Fig. 2).

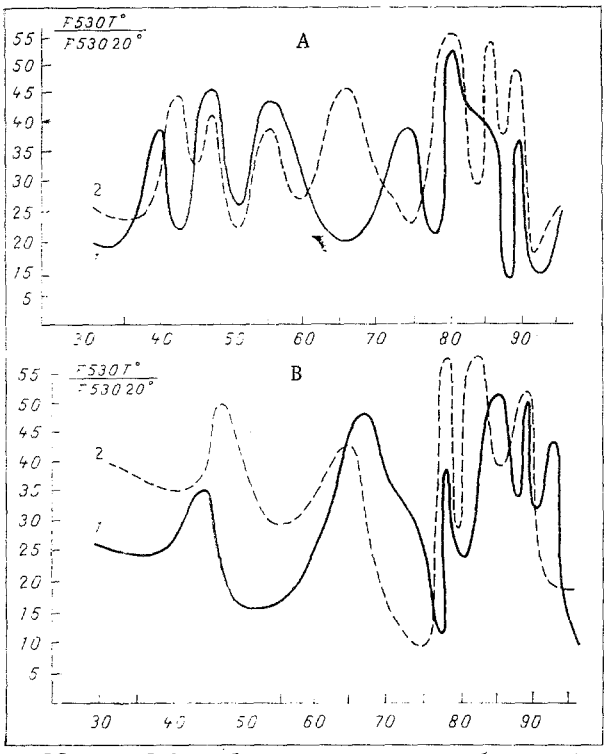

Fig. 2. Effect of homologous serum on chromatin melting curves of human peripheral blood cells (F_{530}). A) Healthy human cells (lymphocytes and granulocytes): 1) incubated in autologous serum (10%); 2) incubated in serum of patient with CML in phase of blast crisis; B) cells of patient with CML in phase of blast crisis: 1) incubated in autologous serum (10%); 2) incubated in healthy human serum (10%).

To sum up the results of this investigation it will be noted that previously the writers discovered healthy individuals with "nonstandard" chromatin melting profiles [3], which were placed in a special subgroup with "unclassified" cases (about 8%). On the basis of their chromatin melting profiles, patients with CML in the blast crisis phase fell into the "unclassified" subgroup in 100% of cases.

In our view an increase in the resolving power of the method used will allow further classification of the so-called "nonstandard" curves and will reveal correlation between them under normal conditions and in the corresponding pathology. This is very important, because the solution to this problem may enable the phenomenon we have analyzed to be used as an indication of predisposition to the disease.

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