Special experiments showed that antibody-forming cells in this model were chiefly of recipient origin.

The operation produced the ability of the lymphocytes to induce a GVHR much sooner in the CBA than in the C57BL mice. Accordingly the number of antibody-forming cells was higher in the experimental recipients of CBA lymphocytes 4 h after the operation than in recipients of C57BL lymphocytes.

Virtually no difference in the ability of the spleen cells to induce the GVHR was present in the two strains 17 h after hepatectomy.

These results thus indicate that the lymphoid tissue of CBA mice is more labile and responds more rapidly by the modification of its functional properties and cell proportions characteristic of regeneration of the liver than in C57BL mice.

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REVERSIBILITY OF STRUCTURAL CHANGES IN CHROMATIN OF INTERPHASE NUCLEI OF PERIPHERAL BLOOD LYMPHOCYTES OF PATIENTS WITH DOWN'S SYNDROME UNDER THE INFLUENCE OF HEALTHY HUMAN SERUM

K. N. Fedorova

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A luminescence-microscopic investigation of short-term human cell cultures stained with Acridine Orange showed that the melting curve of the cell chromatin DNA in the region from 78 to 85°C depends on changing external environmental conditions, i.e., on the composition of the blood serum.

KEY WORDS: Down's syndrome; interphase chromatin; lymphocytes; thermal denaturation; Acridine Orange.

A previous luminescence-microscopic study of short-term (1 h) cell cultures stained with Acridine Orange revealed a clear difference between the melting profiles of the chromatin DNA of lymphocytes from healthy persons and patients with Down's syndrome [2, 3]. The melting curves of chromatin from healthy human lymphocytes have two maxima in the region of 78 and 85°C. Lymphocytes of patients with Down's syndrome were found to have one diffuse maximum in the region of 85°C. The absence of a decrease in fluorescence between 78 and 85°C is considered to be due to the greater degree of condensation of certain regions of the chromatin complex in cells with an altered karyotype (47, XY-21 and 47, XX-21).

The object of this investigation was to study the genesis of the following phenomena: Are the changes observed caused by stable disturbances of the structure of the chromatin in trisomic cells (as is indicated in

Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow.

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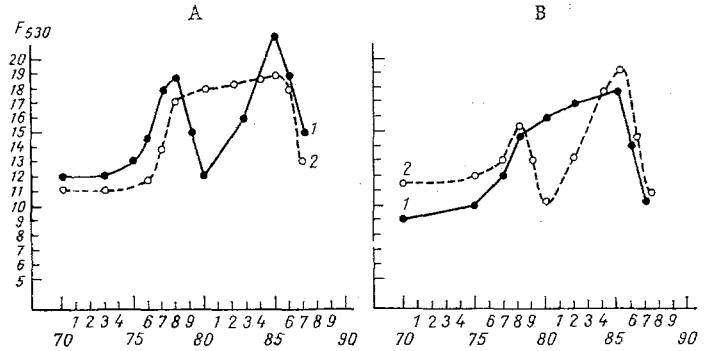

Fig. 1. Effect of homologous serum on melting curves of nuclear chromatin DNA of human peripheral blood lymphocytes within the temperature range from 75 to 90°C, obtained by luminescence microscopy with Acridine Orange (F_{530}). A) healthy human lymphocytes: 1) incubated in autologous serum (10%), 2) incubated in serum of patient with Down's syndrome (10%); B) lymphocytes of patient with Down's syndrome: 1) incubated in autologous serum (10%), 2) incubated in healthy human serum (10%). Ordinate, F_{530} (intensity of fluorescence of dye bound with nuclear chromatin of cells, at λ =530 nm); abscissa, temperature (in °C).

directly by the increased gene dose, i.e., the presence of an extra chromosome) or are they due to the effect of changed components of the cell's external environment, i.e., the blood serum of the affected person?

EXPERIMENTAL METHOD

Melting curves of DNA from cell chromatin from persons with Down's syndrome, whose lymphocytes were preincubated for 1 h in healthy human serum, and vice versa, were obtained.

Altogether 20 experiments were carried out. Material was obtained from ten healthy donors aged 20-26 years and from ten patients with Down's syndrome aged 16-20 years. As the temperature factor, thermal denaturation of the cells was carried out by a method modified by the author [6]. The modification was as follows: 1) the use of formalin as fixative was eliminated as an agent denaturing DNP whose action is difficult to allow for [1]; 2) to prevent renaturation, acetone : ethanol (1 : 1) at −5 and −7°C was used as the fixative, it being calculated that during transfer of the container with the preparations from the incubator to the fixative the temperature of the latter would not rise above 0°C.

The stability of DNA as a component of cell chromatin to the action of heat was investigated at temperatures of between 25 and 100°C at intervals of 2 and 2.5°C and between 70 and 90°C at intervals of 1° (±0.1°). Changes in the chromatin structure directly in the lymphocytes were tested by luminescence microscopy [5], by a method based on the ability of the chromatin DNA of these cells to form complexes with the luminescent label (Acridine Orange). The intensity of luminescence of Acridine Orange bound with the chromatin DNA of the lymphocytes was measured on an MSP-0.5 (Opton) scanning microscope-photometer. Light with λ =365 nm was used for excitation; the intensity of luminescence was measured at λ=530 nm by means of a suitable interference filter.

Details of the apparatus and of the method of working with it, as well as the method of isolation and cultivation of the lymphocytes were described previously [4].

EXPERIMENTAL RESULTS

Melting curves (between 70 and 90°C) of chromatin DNA from cells of healthy persons and patients with Down's syndrome are illustrated in Fig. 1. The dye-binding properties of the chromatin from healthy human cells incubated in the autologous serum possessed two maxima at 78 and 85°C ($P < 0.01$), followed by a return to the initial level. The melting profile of chromatin DNA of the patients' cells, also when incubated in autologous serum within the same temperature range, formed a curve with a single diffuse maximum in the region of 85°C ($P < 0.01$). On incubation of the cells of the same patient in healthy donor's serum (Fig. 1B, 2) the dye-binding properties of the chromatin of these cells were modified, for two maxima appeared in the region of 78 and 85°C ($P < 0.01$), just as with healthy human cell chromatin.

The melting profile of the chromatin DNA of healthy human lymphocytes when incubated in the patient's serum also was changed and consisted of a curve with a single diffuse maximum in the region of 85°C ($P < 0.01$), typical of the cell chromatin of patients with Down's syndrome (Fig. 1A, 2).

The investigation thus showed that the melting curve of DNA of the cell chromatin in the region between 78 and 85°C depends on the altered external environmental conditions, i.e., on the composition of the blood serum.

It can be concluded from the results of this comparative analysis of the structural (and, consequently, the functional) state of the chromatin of human lymphocytes that an altered composition of the blood serum causes reversible changes in chromatin structure, which result in a difference in the degree of dissociation of the nucleoprotein complex.

The most substantial differences between the melting curves of chromatin from normal and trisomic cells were observed within a narrow range of temperatures (78-85°C). This fact suggests that the main differences are connected with one chromatin fraction, structural modifications of which are manifested within precisely this temperature range.

The phenomena discovered can be regarded as important evidence which can help to explain the disturbance of synthesis of marker enzymes unconnected with the extra chromosome in trisomic cells [7]. The effect of the physiological environment on the structural and functional organization of the cell chromatin is thus clearly demonstrated.

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CYTOGENETIC EFFECTS OF CHLOROQUINE IN HUMAN LYMPHOCYTE CULTURES

M. A. Shalumashvili and Ya. A. Sigidin

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Addition of chloroquine to a culture of human lymphocytes at the G_1 stage showed that the compound, in a concentration of 15 μg/ml, does not affect the level of chromosomal aberrations, but in concentrations of 60 and 100 μg/ml it suppresses mitotic activity of the cells virtually completely. By its actions on the G_2 stage chloroquine, in a concentration of 100 μg/ml, significantly increases the number of chromosomal aberrations, but in a concentration of 15 μg/ml it has no appreciable action.

KEY WORDS: chloroquine; chromosomal aberrations; lymphocyte cultures.

Experiments in vitro and clinical investigations in recent years have demonstrated the weak mutagenic action of two substances widely used in the treatment of rheumatism: aspirin [3, 4] and butadione [14]. Other substances used in rheumatology have not yet been studied from this standpoint. Among them, special attention

Clinical Pharmacological Group, Institute of Rheumatism, Academy of Medical Sciences of the USSR, Moscow.

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