EFFECT OF AUTOLOGOUS SERUM ON THE STATE OF THE INTERPHASE CHROMATIN OF THE LYMPHOCYTES IN DOWN'S SYNDROME

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A luminescence microscopic study of a short-term culture of human lymphocytes with the aid of acridine orange showed that the modified state of the interphase chromatin in patients with Down's syndrome can be partly restored to normal by incubating the patient's lymphocytes with healthy human serum. These results suggest that the component inhibiting chromatin activation is absent or qualitatively changed in the serum of patients with Down's syndrome. The incomplete restoration of the ability of the chromatin to fix the dye points to irreversible changes in the structure of the chromatin itself, in the direction of condensation.

KEY WORDS: Down's syndrome; interphase chromatin.

A previous investigation [3] by luminescence microscopy with acridine orange showed that the interphase chromatin of lymphocytes of patients with Down's syndrome in short-term (incubation for 1 h) cell cultures differs structurally from healthy human chromatin. This phenomenon is expressed as a spontaneous increase in the intensity of fluorescence of the dye, absence of the activating action of phytohemagglutinin (PHA), and a significant decrease in the intensity of fluorescence of the dye during the first minutes of incubation of the cells. It has also been shown [2] that dilutions of autologous serum in the incubation medium, by contrast with normal, did not cause additional changes in the dye-binding properties of the nuclear chromatin of patients with Down's syndrome. It was postulated on the basis of these observations that the component in the serum of these patients repressing spontaneous activation of chromatin is absent or qualitatively changed, or the structure of the chromatin itself is modified.

Proof of quantitative changes in the serum components of patients investigated has been published in several papers [5-7] the authors of which discovered a marked increase in the serum immunoglobulin level of these patients.

Qualitative changes in the serum immunoglobulin fractions of patients with Down's syndrome have been described by Miller and Mellman [4]. These workers showed by immunoelectrophoresis that a more rapid migration of the serum immunoglobulins toward the anode is found in patients with Down's syndrome than with the identical fractions of healthy human serum. They showed with the aid of papain that the Fe fragment of immunoglobulin is responsible for the specific character of the changes.

In the investigation described below the effect of autologous serum on the structure of the interphase nuclear chromatin of the lymphocytes from patients with Down's syndrome was studied.

EXPERIMENTAL METHOD AND RESULTS

The method of crossed incubation of patients' lymphocytes in healthy human serum and incubation of the cells of healthy donors in the serum of patients with Down's syndrome was used. After these manipula-

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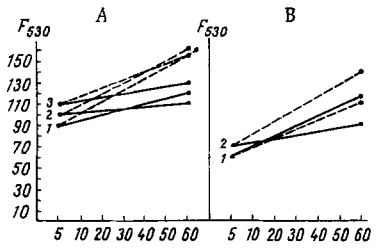

Fig. 1

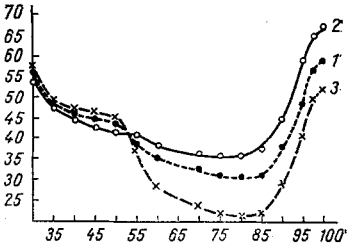

Fig. 2

Fig. 1. Fluorescence of acridine orange bound with chromatin of human peripheral blood lymphocytes at λ-530 nm: A) lymphocytes of healthy donors incubated in serum of patients with Down's syndrome (1), with autologous serum (2), and homologous serum (3); B) lymphocytes of patients with Down's syndrome incubated in autologous serum (1) and in healthy human serum (2). Continuous lines show intensity of fluorescence of dye bound with chromatin without PHA; broken lines show intensity of fluorescence of dye bound with chromatin + PHA. Abscissa, time (in min); ordinate, intensity of fluorescence of acridine orange bound with DNP-complex of lymphocytes.

Fig. 2. Relative length of DNP fibers as a function of temperature: 1) 0.14 M NaCl solution + patient's blood serum in dilution of 1:150; 3) 0.14 M NaCl solution + serum of healthy donor in the same dilution. DNP concentration 0.3 mg/ml, $[\eta]/P = 4.2$, $[\eta] = 32$ dl/g. Abscissa, temperature (in °C); ordinate, relative length of DNP fibers (in percent).

tions a quantitative analysis was made of the pattern of fixation of the luminescent label with the nuclear chromatin of the lymphocytes from 20 patients and the same number of healthy donors. As a first step, to determine the effect of homologous serum on the state of the chromatin of foreign lymphocytes, 15 additional experiments were carried out on 30 donors, in which their lymphocytes were incubated in homologous healthy human serum. This part of the investigation was carried out by cytospectrofluorimetry. The apparatus, the details of the method, and the procedure for isolating and culturing the lymphocytes were described previously [3]. The luminescence of acridine orange, bound with the DNA of the nuclear chromatin of the lymphocytes was used as the test.

This method shows that homologous serum does not affect the ability of the chromatin of healthy human lymphocytes to fix the dye (Fig. 1A). Meanwhile, incubation of lymphocytes of patients with Down's syndrome in healthy human serum led to a significant decrease in the spontaneous activation of the chromatin ($P < 0.02$), and under these circumstances the chromatin could be activated by PHA.

In that case, i.e., during incubation of the patients' lymphocytes in healthy human serum, as is clear from Fig. 1B, the shape of the curves reflecting the change in the qualitative pattern of fixation of the dye was very similar to that in the control experiments. However, this apparent "normalization" of the processes was only partial, for quantitatively the intensity of fluorescence of the dye during the first few minutes of incubation of the patients' cells in healthy human serum did not rise to the normal level.

It is interesting to note that when healthy human cells were incubated in the patients' serum there was a relatively small (5-10%) decrease in the intensity of fluorescence of the dye during the first few minutes of incubation.

To analyze the mechanisms of this "stabilizing" effect thus discovered, a series of experiments was carried out to study the effect of the serum of patients with Down's syndrome on model deoxyribonucleoprotein (DNP) systems in vitro. This series of experiments was carried out by means of the thermomechanical method described earlier [1]. In these experiments model supramolecular systems of DNP were obtained in the corresponding blood serum diluted 150 times with physiological saline. DNP was obtained from calf thymus in 0.7 M NaCl solution. The protein and DNA concentrations in the nucleoprotein were determined by the methods of Lowry and Spirin. The characteristic viscosity of the DNP preparations was measured in a 3-ball low-

gradient viscosimeter of the Ostwald type with gradients (for water) of $\beta = 50\ sec^{-1}$, $\beta = 35\ sec^{-1}$, and $\beta = 23\ sec^{-1}$. The action of the sera was judged by comparing changes in the wavelengths of the DNP tested simultaneously in a physiological medium (0.14 M NaCl) without serum (control), in a medium containing a patient's serum (dilution 1:150), and in medium containing healthy human serum (1:150). Altogether 25 experiments were carried out with the patients' blood serum and 20 with healthy human serum.

As Fig. 2 shows, within the temperature range 50-65°C a marked decrease in the chemical flow of the DNP-fibers kept in medium with serum of patients with Down's syndrome compared with medium containing healthy human serum was observed. These changes were not connected with the activity of nucleases or proteolytic enzymes, for their inhibition did not abolish the differences discovered.

At 25°C and in the interval 82-97°C (the region of helix-coil transition of DNA in DNP) no significant changes were observed in the parameters of the DNP fibers.

The results thus indicate that the modified state of the interphase chromatin in patients with Down's syndrome can be partly restored to normal. It can be concluded from analysis of the information thus obtained that at least two processes leading to the manifestation of the combined pathological effect exists: the first is connected with a change in the chromatin and is induced by the blood serum of patients with Down's syndrome. This change could perhaps be due to the absence or qualitative modification of the component of the blood serum that inhibits the spontaneous activation of chromatin. The presence of a stabilizing action of the serum of patients with Down's syndrome both on the nuclear chromatin of healthy human lymphocytes in vivo and on fibers of DNP systems in vitro is further confirmation of the presence of modified components in the patients' serum.

The second process may perhaps be linked with irreversible changes in the structure of the chromatin in Down's syndrome (condensation). The incomplete restoration of the ability of the chromatin to fix the dye after incubation of lymphocytes in healthy human serum points to the possibility of such a mechanism.

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