ANALYSIS OF CHANGES IN THE NUCLEAR CHROMATIN OF PERIPHERAL BLOOD LYMPHOCYTES IN DOWN'S SYNDROME

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Experiments with human lymphocytes incubated for 1 h in autologous serum in various dilutions, using a method of luminescence microscopy with acridine orange, showed that a gradual decrease in the concentration of autologous serum in the incubation medium led to a corresponding increase in the intensity of fluorescence of the dye bound with DNA (chromatin) in healthy human lymphocytes. This phenomenon did not occur in the lymphocytes of patients with Down's syndrome; i.e., qualitative differences are present. The author postulates the absence of or a qualitative change in the component repressing chromatin activation in the serum of patients with Down's syndrome.

In a previous investigation [1] an attempt was made to demonstrate functional changes in the chromatin of lymphocytes from patients with Down's syndrome experimentally. These changes, revealed with the aid of the dye acridine orange (AO) in a cell culture incubated by a modified Moorhead's method, took the form of a spontaneous increase in the intensity of fluorescence of dye bound with DNA (chromatin) and the absence of the activating effect of phytohemagglutinin (PHA).

At the same time as these investigations were proceeding, other workers [3, 4], using Giemsa's and quinacrine mustard staining methods, revealed structural changes in the chromatin of the lymphocytes of patients with Down's syndrome, expressed as an increase in the number of heterochromatin regions in the chromosomes of these patients compared with healthy subjects.

Facts have thus been obtained from which it can be concluded with a fair degree of certainty that the interphase and metaphase chromatin of lymphocytes of persons with a modified karyotype (XY 47) differs both structurally and functionally from healthy human chromatin.

The object of this investigation was to continue the analysis of the function of the nuclear chromatin of cells with a chromosomal anomaly and also to determine the relative importance of the chief participants in the pathological reaction (the nuclear chromatin and the serum of the external medium) in the manifestation of the combined pathological effect.

EXPERIMENTAL METHOD

Cytospectrofluorimetry was the chief method of investigation used [4]. The apparatus, the details of the work, and methods of isolating and cultivating the lymphocytes were described earlier [1]. Luminescence of AO bound with the DNP-complex of the lymphocyte nuclei was used at the test. The results given below were obtained in parallel investigations of the nuclear chromatin of lymphocytes from 30 healthy donors aged from 18 to 30 years and 36 patients with Down's syndrome aged from 8 to 18 years. Nuclear chromatin of the lymphocytes of 10 healthy children aged from 6 to 18 years also was tested. In addition, 16 experiments were carried out to study the intensity of fluorescence of the dye bound with the nuclear

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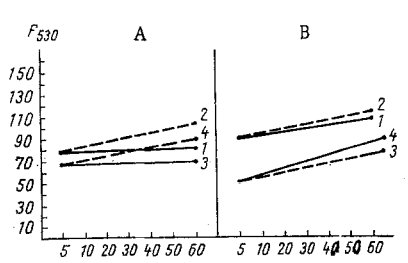

Fig. 1

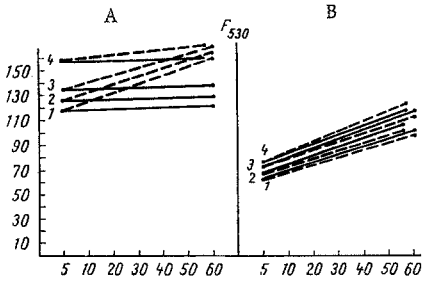

Fig. 2

Fig. 1. Fluorescence of acridine orange bound with chromatin of human peripheral blood lymphocytes (healthy subjects – A, patients with Down's syndrome – B) at $\lambda = 530$ nm. 1) Incubation in 100% autologous serum; 2) incubation in 100% autologous serum + PHA; 3) incubation in 10% autologous serum; 4) incubation in 10% autologous serum + PHA. Here and in Fig. 2: abscissa, time (in min); ordinate, intensity of fluorescence of acridine orange bound with DNP-complex of lymphocytes (F_{530}).

Fig. 2. Fluorescence of acridine orange bound with chromatin of human peripheral blood lymphocytes (healthy subjects – A; patients with Down's syndrome – B) in incubation medium containing different concentrations of autologous serum; $\lambda = 530$ nm. 1) Concentration of autologous serum 0.5%, 2) 0.25%, 3) 0.35%, 4) 0.05%.

DNP-complex of lymphocytes from patients and healthy subjects after incubation of the cells in 100% autologous plasma without the addition of nutrient medium; i.e., under conditions closest to those pertaining in vivo.

EXPERIMENTAL RESULTS

Incubation of lymphocytes from patients with Down's syndrome for 1 h both in 100% and in 10% autologous serum led, by contrast with the control experiments, to an increase in the intensity of fluorescence of the dye on the average by 30 and 35% (Fig. 1A), i.e., spontaneous activation took place. The addition of PHA to the incubation medium caused no further change in the dye-binding properties of the chromatin of the patients' lymphocytes, which contrasted with a considerable (by 40-60%) increase in the amount of dye bound by cells from healthy donors (Fig. 1B).

It is remarkable that transferring the cells from 100% autologous serum to medium containing 10% autologous serum led to a decrease in fluorescence of the dye bound with the patients' chromatin on the average by 50% at the fifth minute of incubation. A similar property also was found in the healthy donors, but to a much lesser degree (20-25%). To study the function of the nuclear chromatin more closely, a further 15 experiments were carried out in which lymphocytes of patients and healthy donors were incubated in autologous serum diluted consecutively with Eagle's medium (three concentrations of serum were tested: 1/25, 1/35, 1/100; Fig. 2). None of the three dilutions of serum gave any additional change in the dye-fixing properties of the nucleo-protein complex in the nuclei of the patients' lymphocytes (Fig. 2A); i.e., the function of the patients' chromatin was unchanged regardless of the dilution of the serum below 10%. The picture was completely different on diluting the serum of the healthy donors (Fig. 2B). Each successive dilution led to an increase in the intensity of fluorescence of the dye after the fifth minute of incubation, while the amplitude of the activating effect of PHA was correspondingly reduced: with the maximal dilution of serum (1/100) all three points tested, i.e., the 5th and 60th minutes of incubation, and also the 60th minute of incubation after the action of PHA give maximal intensities of radiation of the dye, numerically speaking almost identical. No difference was found in the dye-fixing properties of the chromatin in children and adults.

Analysis of the effect of different concentrations of autologous serum on the functional state of the nuclear chromatin from healthy human lymphocytes thus showed that each successive dilution of serum led to a corresponding increase in the intensity of fluorescence of the dye during the first minutes of incubation of the cells. These results are taken as unambiguous evidence of the existence of a factor in healthy human serum inhibiting the template activity of chromatin.

In Down's syndrome none of the dilutions of serum caused activation of the chromatin on the lymphocytes in the initial stage of incubation, suggesting either that this repressive component is absent or its activity is inhibited, or that the chromatin itself undergoes qualitative changes and loses its ability to make contact with the repressive component in the serum.

The low intensity of luminescence of the dye during the first minutes of incubation of the trisomic cells in all dilutions of autologous serum suggests that the lymphocytes of patients with Down's syndrome have abnormal chromatin with increased powers of condensation or, in other words, that the processes of chromatin repression are relatively predominant in these patients by comparison with healthy subjects.

On examination of the general picture of the change in the chromatin of patients with Down's syndrome a situation of a dual character was found to exist: on the one hand, a serum factor inhibiting chromatin activation disappears from the medium or undergoes qualitative changes, while on the other hand the chromatin is repressed during the initial moments of incubation in autologous serum.

At the present level of our knowledge the following explanation can be proposed: the serum factor inhibiting activation is probably a complex consisting of repressive and derepressive components; in Down's syndrome the activating part of this complex is perhaps absent, with the result that the repressive factor is stimulated; i.e., the abnormal part of the chromatin becomes condensed and its unchanged part is activated, giving rise to the phenomenon of spontaneous activation.

These and other possible explanations can be verified in systems providing for crossed substitution of the sera and lymphocytes of patients and healthy subjects in cell culture. It would also be useful to analyze the direct effect of the blood serum of patients and healthy subjects on model systems in vitro.

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