PROPERTIES OF CHROMATIN OF PERIPHERAL BLOOD LYMPHOCYTES OF WOMEN WITH THREATENED ABORTION

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Threatened abortion is a serious condition that may be reflected unfavorably in the state of the fetus or newborn infant. It is therefore extremely important to study this pathological state and to develop methods of its early diagnosis. It has been shown [1, 5] that in threatened abortion the pattern of blast transformation of peripheral blood lymphocytes is modified. We know [9] that during blast transformation of lymphocytes changes take place in the properties of the chromatin, and that they precede or, at least, accompany an increase in its transcription activity. Accordingly, the writers have made a detailed cytochemical study of chromatin in women with threatened abortion.

To judge the properties of chromatin the following criteria were used: accessibility of DNA to low-molecular-weight ligands — acridine organe (AO) and actinomycin D ([^{3}H]-AMD), and ability of histones to be stained with an acid dye after removal of DNA.

EXPERIMENTAL METHOD

Peripheral blood lymphocytes were studied from 249 women, 131 with normal pregnancy and 66 with threatened abortion. Lymphocytes also were studied from 62 nonpregnant women (blood donors). In most cases films were made from a drop of peripheral blood in the usual way and were fixed without drying. In some cases lymphocytes were studied after culture for 5 or 60 min with the addition of phytohemagglutinin (PHA) or without it. For this purpose heparinized blood plasma was diluted with medium No. 199 to a concentration of 10^6 leukocytes/ml and poured into Petri dishes with cover slips lying on the bottom. Incubation was carried out at 37°C. The preparations were removed after definite time intervals. To detect binding of AO and [^{3}H]-AMD with chromatin, the preparations were fixed, without drying, in a mixture of absolute ethanol and acetone (1:1). To obtain comparable results, subsequent procedures were carried out simultaneously. Staining with AO was done according to Rigler's method [9] with certain modifications [3]. Some fixed films were treated with [^{3}H]-AMD by the method described previously [8]. The quantity of [^{3}H]-AMD bound with DNA-protein was determined autoradiographically.

The relative DNA content was judged from the intensity of fluorescence of the nuclei after Feulgen's reaction in the fluorescence version [6]. The optimal time of hydrolysis (8 min) was determined from the hydrolysis curve. The ability of histones in the lymphocyte nuclei to stain with acid dye was determined by fluorescence microscopy, using primuline, after removal of DNA [4].

Binding of fluorochrome with the nuclei was judged from the intensity of green fluorescence (I_{530}), which was determined cytofluorometrically and expressed in conventional units.

EXPERIMENTAL RESULTS

Binding of AO and [^{3}H]-AMD with chromatin from the lymphocytes of women with normal pregnancy and with threatened abortion is shown in Table 1. Clearly, binding of AO and [^{3}H]-AMD was reduced equally in the lymphocytes of women with threatened abortion and of women with

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TABLE 1. Cytochemical Characteristics of Lymphocyte Chromatin (M ± m)

Cytochemical Parameter	Normal pregnancy (N)	Threatened abortion (A)	Differences: (A − N)/N, %
Binding of [^{3}H]-AMD	Number of grains of silver above nucleus in lymphocytes		
	16.9 ± 0.3	14.6 ± 0.3	14*
Binding of AO	Intensity of fluorescence		
	217 ± 2.5	184 ± 4.0	15*
Binding of primuline after removal of DNA	120 ± 2.5	134 ± 3.0	12*
Binding of rivanol after Feulgen hydrolysis	141 ± 2.3	145 ± 3.1	3

Legend. For first parameter each value is mean for 90 cells from 3 women, for second parameter — from 25 women, for third and fourth parameters — from 12 women. *P < 0.05.

TABLE 2. Changes in Binding of AO by Lymphocytes of Women during Different Stages of Pregnancy, Normal and Complicated by Threatened Abortion, during Culture for 5-60 min with or without PHA (M ± m)

Terms of pregnancy	Intensity of binding of AO, I_{530}, relative units							
	normal pregnancy, culture			n	threatened abortion, culture			n
	5 min	1 h	1 h + PHA		5 min	1 h	1 h + PHA	
I	203±3,1	207±2,4	288±4,8	24	175±2,1	205±3,1	211±3,4	34
II	193±9,3	192±3,3	266±4,1	25	155±3,5	189±3,7	200±4,1	27
III	171±4,8	180±4,3	268±5,4	12	147±2,5	173±3,1	167±3,4	15

Legend. n) Number of subjects tested.

TABLE 3. Binding of AO by Lymphocytes of Women with Normal Pregnancy (M ± m)

Normal blood donors				Normal pregnancy			
5 min	1 h	1 h + PHA	n	5 min	1 h	1 h + PHA	n
160±4,3	167±3,2	236±4,5	62	145±3,1	153±2,4	215±5,3	57

Legend as to Table 2.

normal pregnancy. Parallel with these changes in the chromatin of lymphocytes of women with threatened abortion there was an about equal increase in the intensity of staining of histone amino groups with primuline (an increase of 12%). Measurement of the DNA content (Table 1) showed that lymphocytes of women with normal pregnancy and with threatened abortion are indistinguishable according to this parameter. It is thus evident that we are dealing with a true change in the properties of the chromatin. In the modern view [2, 11], such changes in the properties of chromatin are based on intensification of interaction of DNA with histones in the DNA–protein complex, and these changes are evidence of a more regressed state of the cell chromatin [10]. The opposite trends of binding of AO and primuline in the lymphocytes in this pathology agree with data obtained by a study of the properties of DNA-protein of cells during maturation [4] and spermatogenesis [7]. The principles of this phenomenon require further study.

It was interesting to study the character of changes in the properties of lymphocyte chromatin from patients and normal women under the influence of treatment of the cells with PHA. As the criterion for judging the properties of chromatin, binding of AO was used. The results are given in Table 2. Clearly binding of AO by lymphocyte nuclei of women with normal pregnancy decreased in the course of its development and was unchanged during culture for 1 h. Addition of PHA to the incubation medium led to an increase in the intensity of fluorescence of AO in the lymphocytes of women with normal pregnancy at different terms by 38-48%.

After incubation of lymphocytes from women with threatened abortion for 5 min a decrease in the intensity of fluorescence of the bound dye by 16-24% was observed compared with normal pregnancy. Culture of lymphocytes for 60 min without PHA caused an increase in the intensity of binding of AO by the cell nuclei in threatened abortion.

The results of the study of binding of AO by lymphocytes from women with normal pregnancy are given in Table 3. On incubation for 1 h, binding of AO by the lymphocytes was not significantly changed either in the normal blood donors or in women with normal pregnancy. Under the influence of PHA, binding of AO was significantly increased in both cases (by 40%). During culture for 5 min binding of AO in lymphocytes from women with normal pregnancy was significantly reduced compared with normal blood donors.

This cytochemical study thus showed that lymphocyte chromatin from women with normal pregnancy is in a more derepressed state than lymphocyte chromatin from nonpregnant women (blood donors). Lymphocytes from women with normal pregnancy are activated by PHA just like blood donors' lymphocytes.

In threatened abortion an even greater degree of derepression of the peripheral blood lymphocyte chromatin is found. When these lymphocytes are cultured *in vitro* they undergo spontaneous activation, as a result of which, probably, addition of PHA in this case does not lead to increased AO binding by chromatin.

The higher degree of repression of lymphocyte chromatin from women with threatened abortion compared with women with normal pregnancy was detected by three independent methods and would appear to be reliable. The data in this paper demonstrate the prospective value of cytochemical tests of the properties of chromatin for the diagnosis of threatened abortion and for monitoring its course.

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